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JOHNS HOPKINS UNIVERSITY,

BALTIMORE.

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STUDIES

FROM THE

BIOLOGICAL LABORATORY,

EDITOR:

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VOLUME II.

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STUDIES FROM THE BIOLOGICAL LABORATORY

OF THE

JOHNS HOPKINS UNIVERSITY.

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A CONTRIBUTION TO THE STUDY OF INFLAMMATION AS ILLUSTRATED BY INDUCED KERATITIS. By WILLIAM COUNCILMAN, M.D. (Plate IV.)

(From the Biological Laboratory of the Johns Hopkins University.)

It would be useless to attempt to give here any but the briefest sketch of the views which have been held concerning the origin of pus and the nature of the cellular changes occurring in inflammation since the time of the establishment of the cell theory. A mere recapitulation of the articles written on this subject in the decade of '60—'70 would fill pages. I will, however, briefly glance at some of the more important ideas which have been or are held with reference to it.

Rokitansky was one of the first to appear in this field of literature. He, in accordance with the cell theory of Schwann (that is, the free cell formation doctrine), assumed that the pus corpuscles were formed in the exudation which played the part of the blastema. These ideas prevailed generally until 1855, when Virchow was led, from his knowledge of the connective tissue corpuscle, to dispute the free cell formation, and to apply the law " *Omnis cellula e cellulâ* " to pathological new formations. Virchow held that the pus cell was the direct derivative of the connective tissue corpuscle, because, wherever he found pus he also found connective tissue in some of its forms ; and since he was obliged from his views to have some cell as the parent of the pus cell, he took the connective tissue corpuscle.

Stricker appears as the latest, and certainly the ablest, defender of these views, though they have undergone essential modifications at his hands. He says that the cells of a tissue, when inflamed, return to their former undifferentiated embryonic condition, become amœboid, and possess the power of dividing indefinitely. This property holds good for all tissues equally ; no matter whether muscle, gland, or ganglion cell, they all can undergo this change and become converted into pus. He holds also that, when the cells return to the embryonic condition, they again become capable of differentiation, and that blood vessels, and even

blood corpuscles, are formed in an inflamed part in the same manner as
in the embryo. Against these views we have what is known as the
" wandering cell " theory, which assumes that the pus cells are white
blood corpuscles which have escaped from the vessels. Waller had, as
early as 1848[1], observed the passage of the white corpuscles through the
walls of the vessels. At that time his observations attracted but little
attention, and were generally distrusted. Cohnheim in 1868[2] firmly
established the fact that the white corpuscles did pass through the vas-
cular walls, and, as the result of his study of the inflammatory processes
in the frog's cornea, tongue, and mesentery, asserted that the pus cells
are white blood corpuscles.

From his study of keratitis, principally induced by cauterizing the
centre of the frog's cornea with silver nitrate, he found that, however
great the number of pus cells in the inflamed tissue might be, the fixed
corneal corpuscles with their processes were unchanged ; that the nuclei
of the corneal corpuscles did not increase ; that the clouding of the
cornea always began at the periphery and from there advanced to the
centre ; that after the injection of pigment granules into the blood some
of the pus cells in the cornea were found with similar granules in their
bodies. From these four circumstances, supported by the direct know-
ledge that the white corpuscles in inflammation did escape through the
vessels in large numbers, he concluded that the pus corpuscles were not
derived from the fixed cells of the cornea, but had wandered in from
without. Stricker, as the result of observations made on the frog's
cornea and on the cornea of the cat, asserts that the three first argu-
ments are based upon imperfect observations, and that the conclusion
formed from the fourth is illegitimate. According to Stricker, the
fixed corpuscles do undergo change, their nuclei increase, and the
clouding always begins where the injury was inflicted. With regard to
the presence of pigment-bearing pus cells in the inflamed cornea after
the previous injection of pigment into the blood, he thinks the granules
could have passed through the walls of the vessels as easily as the blood
corpuscles and have been carried by the lymph streams into the cornea.
There they could easily have been taken up by pus cells which were
already produced by multiplication from the corneal corpuscles.

Here I may remark that the passage of solid dead particles through
the walls of a blood vessel without being *carried* through by the white blood

[1] *Phil. Mag.*, Vol. xxix.
[2] Virchow's *Arch.*, Bd. xl.

corpuscles, easily as Stricker thinks it could happen, has up to this time been seen and described by no one. That Cohnheim's description does not hold good for all cases of induced keratitis, even on the frog's cornea, is certain; but the differences can be easily reconciled. Stricker bases nearly all his views of inflammation and of inflammatory new formations on his study of keratitis. I shall, I think, be able to show in this paper that these views, certainly as far as keratitis is concerned, are erroneous, and may possibly be due, even in his case, to imperfect observations.

I can only excuse my temerity in entering upon a field of research in which so many and distinguished investigators have laboured, by the fact that when endeavouring to satisfy myself of the correctness of Stricker's views on the subject, I obtained, after nearly a year's steady work, results which lead to conclusions utterly at variance with his, but which I think go far towards clearing up some of those points in the pathology of keratitis over which there has been most contention.

The corneas of the frog and of the cat have been principally used in my investigations; the latter animal being chosen for studying the processes in the mammal from the advantages its cornea offers over many others for investigation, especially in the readiness with which it can be split into layers.

The structure of the normal cornea has been thoroughly investigated by various observers in recent years. We know that its proper tissue is lamellated, and consists of flattened branched cells embedded in inter-communicating centres (the serous canaliculi) hollowed out in an inter-cellular fibrillated ground substance, which makes up the larger portion of the corneal mass; that the tissue is well supplied with nerves arranged in plexuses which become finer towards the conjunctival surface; that with hæmatoxylin or gold the cells stain and are seen to communicate by their branches; and that with silver nitrate the ground substance is tinted, while the cells and cell spaces are left unstained. Hæmatoxylin also stains the nerves, while with silver preparations the lymph channels in which the larger ones run are seen as colourless lines.

We also find, even in the normal cornea, another set of cells, which cannot be considered a part of its fixed histological elements. Their numbers are variable; in some corneas very abundant, in others few: in animals of the same species sometimes they are found in greater numbers at one portion of the tissue, sometimes at another. In fresh preparations they can be seen to pass by active amœboid movements from one place to another, and they never, so far as we can see, stand in any fixed his-

tological relation to the other elements of the tissue; these are the " wandering cells." Their position is not at all constant; sometimes we find them lying in the cell space along with the branched corpuscles, sometimes in the narrow communication between two spaces, sometimes as long drawn out rods in the tissue between the fibres (b, Fig. 1, Pl. IV.), sometimes in the nerve lymph channels, and in one preparation I have been so fortunate as to get one seemingly in the act of passing from the nerve channel into a cell space communicating with this, half of its body lying in the channel and half in the space. They can be clearly distinguished from the branched corpuscles both in the fresh condition and when stained; they are much smaller, and with the usual reagents they stain more brilliantly than the others. In fresh preparations in aqueous humour they are easily recognized by their amœboid movements, their greater index of refraction, and their granular contents.

So much for the normal cornea. We will now take up the pathological changes which occur after an acute keratitis has been induced, commencing with those seen in the frog's cornea.

I have employed various means for exciting inflammation here The passing of a thread through the centre of the cornea and bringing it out through the sclerotic, the application of various caustics, such as croton oil, silver nitrate, caustic potassa, and the hot iron (actual cautery). With few exceptions they produce results relative to the severity of the stimulus used. Agents such as the hot iron, which at once kill the tissues with which they come in contact, will, of course, produce less inflammation in surrounding parts than those like the thread, whose action is more or less gradual. A method which I have used on the frog's cornea with excellent results has been to pass a thread through the membrana nictitans and then make several pricks in the cornea with a needle. The inflammation produced by this method will be discussed separately, since results are in this way obtained which at first seem perplexing.

As one of the most typical, I will take a cornea which has been inflamed by touching it at the centre with a crystal of silver nitrate.

This may be examined after various intervals of time have elapsed, both in the fresh condition and after staining. About twenty hours from the application of the caustic the most important changes can be seen. To examine fresh, it is necessary to puncture the sound eye and collect the aqueous humour on a slide; the inflamed cornea is then carefully excised and spread out in this, with the posterior surface uppermost.

To avoid folds in the tissue it is better to make three or four incisions at the edge, extending for some distance towards the centre, before putting on the cover slip. The powers I have found most satisfactory to use have been the No. 2 immersion of Zeiss ($\frac{1}{11}$) and the E of his dry system ($\frac{1}{8}$).

The first thing noticed here is that the large branched cells are visible ; in the normal they cannot be made out at all directly after the cornea is cut out, and only appear after an interval of half an hour to an hour. They are no more granular than in the uninflamed, and present no changes from the normal an hour after the excision of the latter. Why they become at once visible I do not know ; it may be due to some change in the refraction of the ground substance caused by the greater amount of fluid now in the tissue, or to some change having taken place in the corpuscles and only revealing itself in this way, or to both.

The wandering cells are present in vast quantities, exhibiting the most active and varied movements ; while in the normal cornea, as before remarked, we only occasionally see them. Sometimes one may be seen to send out a long process, at the end of which a knob presently appears, which, growing larger and larger, finally becomes the main body of the cell : as though in this way it had passed from one space to another through a narrow communication. Sometimes we see them as more or less irregular bodies, undergoing changes of form and not of position ; again as the long, staff-like bodies spoken of in the normal cornea. They are present in the greatest numbers at the edge, becoming fewer as we proceed to the centre. Since in the fresh specimens our observations must be made on the whole thickness of the cornea, all these changes become much more clear and can much better be studied after it is stained and split up.

For staining I always use the double staining in silver with hæmatoxylin or carmine, the former being much preferable for the frog. The cornea is exposed by pushing the eye upward from the roof of the mouth, and rubbed smartly with the solid crystal of silver. At the expiration of ten minutes it is cut out, and exposed in glycerine to the action of diffused daylight ; when it becomes of a light brown colour it is split up and stained in one of the two reagents mentioned. With care the cornea of the frog can easily be split into eight or nine layers. I vastly prefer this method of staining to the gold chloride method, which has hitherto been almost exclusively used in these investigations. It has the great advantage of being always certain in its results ; while

gold, although sometimes giving us beautiful preparations, is the most uncertain of reagents, and its success depends for the most part on unknown circumstances. Another great advantage is that we have both the negative and the positive picture at once ; the cell space shown with the cell within, and the relation of the one to the other always is kept in view. The preparations are mounted in slightly acidulated glycerine.

In preparations of the twenty-hour cornea examined after this treatment we can easily make out three distinct parts :—A central one, on which the caustic was applied, and which is now represented by a black scar, in which the cell spaces are imperfectly seen. Around this is a zone of variable width, in which absolutely no change from the normal can be made out ; here we see the sharply-defined cell space, with the nucleus, or, in deeper staining, the body of the cell within. The width of this zone is dependent on the extent of the injury, the length of time which has elapsed since its infliction, and on the general irritability of the tissues of the animal used. Without doubt, from the same amount of irritation, the extent of the pathological changes in some animals of the same species is different from that seen in others.

This zone passes, separated by no well defined line, into the outermost one. In this, along with the corneal corpuscles, other elements can be seen in numbers far in excess of the branched cells and always in the greatest quantity at the outer edge. These other elements stain in all respects similarly to, and are always of the same size as, the wandering cells previously described in the normal cornea. They can always be distinguished from the branched cell, even when lying in the same cell space with it. In one place we see the nerve channel filled with them, in another we see them lying in the tissue between the fibres, and elongated until they have the appearance of rods. Again we see them in the cell spaces or in the narrow interspace between two cells ; their form always influenced by the dimensions of the cavity in which they lie. Often where they are most numerous in the tissue the branched corpuscles cannot be made out at all. It may be that these are simply concealed by the vast numbers of the others, or it is possible that the fixed corpuscles have then been absorbed or destroyed by the young and vigorous strangers.

In no case do we see in the corneal corpuscles proper, any indications which would lead us to suppose that multiplication had occurred or was taking place. They stain with reagents as did the normal, and the nucleus always has the same shape as this, except in instances where it

may be indented by pressure from a wandering cell lying in the same space, as represented at a, in Fig. 1, taken from the normal cornea. If the cornea be examined at an earlier period, say twelve hours after the injury, these wandering cells will be confined to a small area at the outer edge ; if later than twenty hours, forty, for example, they will be found to fill almost the entire cornea, completely obliterating the unchanged zone in some cases.

If we examine the surrounding portions of the sclerotic and conjunctiva we find the blood vessels full of cells just like these, and the whole tissue there also infiltrated with them.

A still further proof that these wandering cells enter the cornea from without is furnished by the result of the injection of finely divided colouring matter into the blood, according to the method of Cohnheim, whose results in this respect I can completely confirm.

If the cornea is cauterized shortly after the injection of cinnabar into a lymph sac or the anterior abdominal vein and examined after the usual time, we find among the wandering cells a great many in which pigment granules are plainly visible, though they differ in no other respect from the others. Sometimes a few granules can be seen in the tissue not inclosed in the cells. These may be accounted for by supposing that they were here dropped by the wandering cell which brought them from the vessel. Stricker himself says that he and Norris have seen one wandering cell transfer to another cell of the same nature some of the vermillion granules contained in its substance. Since the vermillion granule can in nowise contribute to the nutrition of the cell, and forms rather a heavy load to be carried round, we can see excellent reasons why the cell would be willing to throw it away. The number of cells containing these granules is far too large to suppose they could have gotten them in any other way than by taking them up in the blood vessels.

The inflammation produced by methods involving a laceration of the corneal tissue gives some results differing from those last described. Here, as in the last case, we see the peripheral portion of the tissue infiltrated with wandering cells ; but we see them also elsewhere. Around the spot where the injury was inflicted we see cells of the same appearance and offering the same variety of form and position as those at the outside, and here narrowing the zone, which in the cauterized corneas we have described as free from them, very materially. How came these cells here ? From the outer edge they could not come, for we have lying between this and the centre a zone which, in the earlier stages of the

process certainly, is free from them. If now we combine both methods of producing the inflammation, and having cauterized two corneas in the centre, we make a prick at the outer edge of the cauterized spot of one, and examine the two after the usual interval of time, we shall find plenty of wandering cells around the laceration in the cornea whose tissue was punctured, and none at the same spot in the other. Only one conclusion is possible, that they have entered the cornea where its substance was broken. This is easily comprehensible, since a keratitis can scarcely be produced in this way without involving at the same time an extended conjunctivitis, and as a consequence of this having quantities of white blood corpuscles in the conjunctival secretion. From this source they could easily enter the tissue where broken.

The results obtained after passing the ligature through the membrana nictitans point clearly to this. Here a violent conjunctivitis is necessarily set up; many blood vessels in the membrane are ruptured and plenty of white corpuscles poured out. As a consequence, in these preparations we have a very large number of wandering cells at the point where the prick was made; in some cases they are so plentiful that everything else is obscured. After the injection of pigment granules these wandering cells also contain them. No change is seen in the branched corpuscle at either place.

Proceeding now to the cat's cornea, we meet here, even in the normal state, some difference from that of the frog. The corpuscles (Fig. 2), are smaller, are more numerous, and the cell spaces communicate by larger passages than in the frog. The brightly-staining wandering cells in the normal cornea are fewer in number than in the frog's cornea, and mostly found in the cell spaces. Their special characteristics will be described when we come to speak of the pathological changes.

As a means of exciting inflammation I have, following Stricker, used the solid stick of caustic potassa, and found it vastly superior to any other agent. A young cat is preferable to an old one, from the fact that the cornea of the former is much more easily split into its lamellæ than that of the latter. The animal is first etherized and the cornea touched with the caustic; particular care must be exercised in doing this, as the potassa melts so rapidly on contact with the moist surface that there is great danger of its involving too great an extent of tissue. To avoid this the caustic stick must be pointed (which is easily effected by holding it in a stream of water), and the cornea previously carefully dried with filter paper. By varying the period of contact, an eschar

extending only a few lamellæ in depth or one involving the whole thickness of the tissue can be produced. The animal is then left in quiet and the cornea cut out and examined after periods of from 14 to 60 hours. The silver staining, before removal, and the after staining, with carmine, are used. If we examine such a cornea, say 40 hours after cauterization, and as yet unstained by carmine, the changes found can be divided into two heads. Of these the first will comprise the changes around the outer corneal edge, and the second those in the immediate neighbourhood of the eschar. In the first we find the cell spaces somewhat larger and the communications between them wider than in the normal cornea. Scattered about through the tissue we find the strongly refracting rod-like cells, appearing very similar to those we have seen in the frog. If the silver staining has been very deep we find the silver precipitated in the substance of the corneal corpuscle as well as in the ground substance, leaving a clear unstained nucleus in every space.

In the immediate neighbourhood of the eschar the change is more pronounced, and different from anything we have hitherto seen. These changes are all the more important to us, since it is here that Stricker says the corneal corpuscles are undergoing the most rapid proliferation. In the silver preparations we see, lying in the coloured ground, groups of small white spaces with dark brown lines separating them from one another (Fig. 4); these groups correspond in shape to enlarged cell spaces. Stricker seems to have confined his observations to this spot, and explains the picture by supposing the corneal corpuscle has here broken up into a number of smaller cells, and that the brown lines mark off the new cell limits.

Let us now see what the carmine staining shows in the two parts. In the outer ring we have (Fig. 3) in each of the slightly enlarged cell spaces the large oval nucleus of the branch cell totally unchanged, and staining in all respects like the normal. In rare cases we find (as is also the case with the normal) two of these nuclei in a space. In addition to these there are other cells, which, from their characteristic appearance, merit a more detailed description. These have a difference in shape according to whether they are found in the cell spaces and nerve channels, or in the proper corneal substance, there lying between the fibres. In the former they are round, with a brightly-stained granular nucleus of the shape of a horseshoe, and correspond to the wandering cells in the normal cornea. Under high powers (800 — 1·000 ×) the apparently single nucleus is usually found to be

composed of three or four small bodies lying in juxtaposition, the mass being always arranged in the shape of a horseshoe.

When lying in the tissue between the fibres they are elongated, and then appear as jointed rods, each joint having the highly-stained granular nucleus. At first sight these rod-like bodies would seem to be entirely different from the round cells in the spaces ; but, on closer inspection, at different places every variation can here be seen, from the slightly elongated cell with a horseshoe nucleus to the long rod-like cell. If we now stain some of the blood of the cat, we find that the white blood cells have a nucleus of this horseshoe shape and stain in all respects like these wandering cells.

Proceeding now from the corneal edge towards the eschar, we come to a region where the corneal corpuscles are wanting, passing on the way through a district where they have taken on changes which will occupy our attention presently. Beyond this line, which can be seen by even a simple lens, the corneal corpuscles are dead—have been destroyed by the caustic. The cell spaces can be seen, most of them much shrunken, but no nucleus in them, or anything which would afford us proof of the presence of a corneal corpuscle. Lying in these cell spaces, but still more in the tissue between them, are seen multitudes of cells before described, at the scleral edge. These cells become more numerous as we proceed, until we reach a territory where the cell spaces are filled with them (Fig. 5). The spaces here are enlarged, and the communications between neighbouring ones are wider ; spaces and communications are all full, and no one comparing these cells with those at the outer (*i.e.*, the scleral) edge can doubt for a moment that they are similar.

Beyond this line of general infiltration the tissue is totally destroyed. By this I mean that not only its living protoplasm is killed, but its physical properties are also altered. Nothing of the cell spaces can be seen, and apparently the wandering cells can make their way no further. At the point of general infiltration the tissue sloughs.

In corneas examined 10 to 14 hours after cauterization this district of general infiltration is wanting ; no wandering cells are seen there. In the other district, however, that around the outer corneal edge, the wandering cells are numerous ; sometimes so many will be seen that the faintly-stained nucleus of the branched cell is entirely obscured, the wandering cells filling up the space. From this edge they become fewer and fewer as we proceed towards the centre. The line of corneal corpuscles marking off the portion of the cornea in which the corneal corpuscles were destroyed by the caustic from that portion of the cornea where the

corpuscles were uninjured, is not now so well seen, as these corpuscles have as yet taken on no change by which we can distinguish them. We readily see, however, even here, where the living tissue ends. Now it is beyond this line that we get from the silver preparations of a later period of inflammation the appearance as though the corneal corpuscles had proliferated. Here were the colourless areas subdivided by brown lines. From this place Stricker's drawing was made, and here he, judging merely from silver staining of corneas, taken always at a fixed time after the cauterization, supposed the proliferation to have been most rapid. Further examination by better methods, and at different periods, after cauterization, shows us that there is here nothing to proliferate. The tissue is as bare of living corneal corpuscles as a sheet of paper. In 48-hour preparations the line of demarcation is more evident and the tissue beyond more infiltrated with cells than in the 40-hour preparations. In all the portion first described, that along the edge of the sclera, no change can be seen in the nuclei of the branched cells. In corneas examined 60 to 80 hours after cauterization, that portion of the tissue surrounded by the infiltration is converted into a slough, which easily comes away, and the peripheral portions, the district around the sclera, still contain wandering cells.

In the corneal corpuscles which form the line outside the zone of infiltration, and which indicate the separation of the dead from the living proper corneal tissue, we find changes as early as twenty hours after cauterisation. These changes are at this period only shown by a brighter staining ; the whole substance of the cell here stains and elsewhere only the nucleus. At a later period (30 to 40 hours) the nuclei can be seen in different stages of division, and at the same time long processes are sent out from the cells into the dead tissue. These processes become longer (Fig. 6), nuclei pass from the old cell up into them, and thus they form in the dead tissue new corneal corpuscles, but never pus. These processes and new cells stain in all respects like parent cell from which they originated, and the nuclei have the same shape as in the old cells, though they stain more brightly, and are more granular.

The appearance of a segment of the cornea taken three or four days after injury, in which the branched corneal corpuscles are undergoing this proliferation, is most beautiful. The nuclei of the new corpuscles divide rapidly, and in some as many as four can be seen. Even if the whole cornea is destroyed with the exception of a small strip along the outer edge, the corpuscles limiting this take on this renewed activity.

The difference between these two processes—the s u p p u r a t i v e , o n
the one hand, in which the wandering çells are the agents,
and the regenerative, on the other, by which new corneal
corpuscles are produced from corneal corpuscles—is so clear
that no one seeing them side by side could mistake them. In no tissue
in the body can the processes of repair be so clearly studied as in the
cornea ; and in no other tissue can the wandering cell theory as to
the origin of pus corpuscles be so clearly proven to be correct.

DESCRIPTION OF THE FIGURES. PL. IV.

Fig. 1.—Normal cornea of frog, stained with hæmatoxylin. Two of the branched
corneal corpuscles are shown with a wandering cell, *a*, lying in the cell space with one of
them. *b b* represent two of the wandering cells in the substance of the cornea ; these have
taken the elongated form.

Fig. 2.—Normal cornea of a cat, stained with silver and carmine. The ground sub-
stance is stained brown with the silver, leaving the cell spaces unstained. In these are
seen the nuclei of the branched cells stained with carmine. *b b*, two wandering cells in the
cell spaces.

Fig. 3.—Scleral edge of cat's cornea fourteen hours after central inflammation. The
wandering cells, *b b*, are increased in number, and the communications between the spaces
are larger than in No. 2. Silver and carmine.

Fig. 4.—Area of general infiltration forty hours after central inflammation. The cell
spaces are greatly enlarged, and broken up into small areas by the brown silver lines.
The ground substance is reduced in amount, in some places represented only as small
islands.

Fig. 5.—Innermost limit of area of general infiltration. Here, as in No. 4, the cell
spaces are greatly enlarged, and divided into small areas, in each of which the brightly-
stained horseshoe nucleus is seen. From this point to the centre no cellular elements are
found. Silver and carmine.

Fig. 6.—Two corneal corpuscles, which have taken on regenerative changes. The
nuclei have increased in number, and long processes which are much branched have
grown out from the parent cell.

SOME FURTHER OBSERVATIONS ON HEAT-DYSPNŒA.

By CHRISTIAN SIHLER, M.D., *Assistant in the Biological Laboratory, Johns Hopkins University, Baltimore, Md., U.S.A.*

In an article published in Vol. II., No. 3, of this Journal, on so-called heat-dyspnœa, I showed that the increase in the number of respirations which an animal presents when exposed to a warm atmosphere, and while its temperature went up, was principally due to peripheral influences; but I further stated that the heated blood might also act directly on the centres in the medulla, though, if so, producing less effect than the peripheral stimuli.

In the following short communication, I propose to give further support to the first statement, and discuss the second as well as another which I touched in the published essay, namely, the action on the medulla of higher temperatures than those used in my former investigations.

I feel the more inclined to add further proofs to support the conclusions which I reached, as views contradictory to them and based on Goldstein's experiments, which I have shown to be imperfect and inconclusive, are taught in several text books of Physiology, and are gaining ground in the medical profession.

Foster says, on page 377, 3rd Edition: "If the blood in the carotid artery in an animal be warmed above the normal, dyspnœa is at once produced. The over-warm blood hurries on the activity

of the nerve cells of the respiratory centre, so that the normal supply is insufficient for their needs. The condition of the blood then affects respiration by acting directly on the respiratory centre itself."

Fick says, on page 266 of his Physiology, 2nd Edition : " If an animal is artificially heated several degrees above its normal temperature, the respirations become deeper and very much more frequent, even if the quality of the blood is in nowise changed ; yes, even when by energetic artificial inflations arterialisation of the blood is insured ; indeed, it is quite impossible in an animal thus super-heated to produce the state of apnœa. That reflex influences do not come into play here—*e.g.*, from the heated skin—can easily be proven by the following experiment. By application of the proper apparatus one can succeed in heating nothing but the blood flowing in the carotid arteries. As soon as that takes place the frequency of the respirations rises just in the same way as if the whole animal had been heated. From that one must conclude, that it is the increase of the temperature in the respiratory centre itself which increases the irritability, and at the same time diminishes the resistance, so that the exciting agent produces in the same unit of time deeper and more frequent respirations."

In an article on Progressive Pernicious Anæmia, by Herbert Jones, published in the *Practitioner*, February, 1880, we read this : ' Heat is also a stimulant to the respiratory centre in the medulla oblongata, by which the movements of respiration are regulated, and as•Fick and Goldstein have shown, when warm blood is supplied to this centre the respiratory movements become quicker and deeper until marked dyspnœa takes place, although the blood which is circulating in the rest of the body still retains its normal temperature."

I let Exp. 1, see Table I., precede the remarks which I wish next to make. The observation it records, like the rest of my experiments, was carried out on a dog. The temperatures, during my observation, were taken in the rectum or vagina.

I had various reasons for undertaking this experiment. In the former investigation I had found that one animal might breathe 200 to 300 times a minute without its temperature going up, and *vice versá*, the temperature of another animal might go up several degrees while the respirations went up from 26 to 62 per minute only, the cord in the latter being divided in the lower cervical region.

Table I.

No. of observation.	Time.	Temp. to which animal is exposed.	Temperature of animal.	Respirations per minute.	
					WEDNESDAY.
1	10.05	24	38·9	28	Tracheotomized.
2	40	41			Head only in apparatus, breathing warm air through tube.
3	48	44	38·9	86	
4	53	42	38·9	120	
5	58	44	38·9	132	
6	11.05	44	39	200	
7	10	46	39	204	
8	17	48	39	268	
9	20	48	39·1	280	Panting.
10	29	49	39·1	280	
11	35		39·1		Artificial respiration for 2 minutes with cool air, apnœa for ¼ minute ; shallow respiration for 1 minute ; out of apparatus.
12	40	50			
13	45				Artif. resp. for 2 min. ; no apnœa.
14	55		39·1	216	
15	12.30		38·9	37	
16	42		38·9	30	
17	44				Artificial respiration ; apnœa of 1½ minute.
18	1.00		39	36	
19	1.01				Placed in apparatus ; head free.
20	06	35	39	152	
21	11	37	39	240	
22	15	38	39		Artif. respiration for 2 minutes ; no apnœa.
23	20	37	39·1	228	
24	25	38	39·2	310	Panting.
25	30		39·3		Artif. respiration for 2 minutes ; no apnœa.
26	31				Taken out of apparatus.
27	34		39·3	280	
28	3.15				Cord cut.
29	4.27	50	37·6	18	Placed in apparatus ; head and arms free.
30	5.10	58	37·8	18	
31	25	49	38	20	
32	30				Artif. respiration ; apnœa of over 1 minute.
33	47	60	38·5	18	
34	57	60	38·9	22	
35	6.21		39·5	24	

No. of observation.	Time.	Temp. to which animal is exposed.	Temperature of animal.	Respirations per minute.	
					WEDNESDAY.
36	6.23	60	39·5		Artificial respiration ; apnœa of ¾ minute.
37	36	60	39·7	24	
38	46	60	40	21	
39	58		40·1		Artif. resp. for 2 min. ; apnœa over 1 min.
					THURSDAY.
40	9.20		37		Artif. resp. of 2 min. ; apnœa of 1½ min.
41	25	60	37	19	Placed in apparatus.
42	10.30	63	40·8	26	
43	35				Artificial respiration ; apnœa of ½ minute.
44	38	55	41·4	40	
45	43				Artificial respiration ; apnœa of ½ minute.
46	47	50	42	100	Begins to pant.
47	50	49	42·5	156	
48	55	49	42·5		Artificial respiration ; no apnœa.

These facts being brought out on different animals, one object here was to try one and the same animal. That is, to take account of increased temperature, if there was any, and increased number of respirations before the cord was cut and after the cord was cut in the same dog. It will be seen that the present results agree with the former conclusions. The same dog is made to breathe 240 times a minute (Obs. 21) while having a temperature of 39 (38·9 had been the temperature of the dog when the experiment began). When we look, however, for the respiratory rate at the temperature of 39, after the dog's cord had been divided, we find it (Obs. 34) 22. And if the objection were to be made that the dog was unable to breathe rapidly on account of the section of the cord, by looking towards the

end of the table it will be found that this is not the reason, for the dog can make as many as 156 respirations in a minute.

We see, then, that in the same dog, when exposed to warm air acting on a large surface, connected by afferent nerve paths with the medulla, the respirations may go up enormously without the animal's temperature rising ; and, on the other hand, the respirations may go up less than 25 per cent., while the temperature increases over one degree Celsius ; in this latter case the greater part of the skin being thrown out of nervous connection with the medulla by previous section of the cord.

It will further be observed, by glancing over the table, that artificial respiration was carried on several times. Some of these produced apnœa, others did not. At 12.44 (Obs. 17), while the animal was at 38·9°, and not in the warming apparatus, apnœa of $1\frac{1}{2}$ min. was produced. At 1.15 (Obs. 22), while the animal was at 39°, *i.e.*, only 0·1° higher than before (practically not higher at all), the same amount of inflation was not successful. That the 0·1° of temperature was not the cause for this condition is shown further on. When the cord had been cut apnœa was successfully produced, although the temperature of the animal had risen not 0·1°, but 1·1°. Again, when finally (Obs. 47) the temperature had reached 42·5, and the respirations 156, the efforts at producing apnœa were again fruitless. It is clear from this that it is not the temperature of the blood *per se* which makes apnœa impossible. We see apnœa may be possible both at normal and at elevated temperatures ; it may also be impossible both at normal and at elevated temperatures; the reason of the difference being that the dog cannot be made apnœic if he pants vigorously. Of course there is a limit when artificial respiration will at times be successful and at times not : just when the respiration *begins* to grow rapid and take on the character of panting, as is shown in Table II., when the dog had the head only in the apparatus. Here, then, we have another support for our conclusions. In the last paper it was shown that it was not the heat acting on the centres which produces this condition of the animal, in which it cannot be made apnœic. The present observations show the other side of the same fact, and make it evident that peripheral influences, due to exposure of the skin only, may be so strong that they do not allow the centre to come to rest, although there is no venosity of the blood to act as a stimulus, nor has the animal's temperature risen more than a degree.

In the third place, it will be seen (Obs. 46—48, Table I.), that the dog did commence to pant—with the cord cut—after he had reached a temperature of 42.

•2

Let me recall now one of the conclusions of my previously-published paper : "The increased respirations . . . . are due to two causes, skin stimulation and warmed blood." A somewhat closer consideration makes it evident that the experiments there given were not sufficient to show that the warmed blood has any direct central effect : for although by section of the cord in the lower cervical region a large part of the skin was thrown out, yet the fore limbs, neck, and sensitive head, mouth,* and tongue remained in connection with the medulla ; and although in the experiment the direct action of the heated air from without was prevented by keeping the animal's head, &c., out of the warm chest, yet this did not preclude the heating of the nerves of the skin of those parts from within by means of the blood which had been heated in the other parts of the body flowing into them.

To show how sensitive the mucous membrane of the mouth and the tongue is, I add Exp. 2, Table II.

Table II.

December 3rd, 1879.

No. of observation.	Time.	Temp. in apparatus.	Temp. of animal.	Rate of respiration.	
1	7.53	42	39	26	
2	55				Head and fore-feet placed in apparatus.
3	8.01	40	38·9	40	
4	06	40	39	52	
5	08	40	39·1	90	
6	09	40	39·1	152	
7	12	40	39·1	66	Nose free.
8	15	39	39	92	Nose back in oven.
9	16	40	39·1	160	
10	18	40	39·1		Dog pants.

In this experiment it was the aim to have the surrounding air which the animal took into its mouth not very hot, not warmer than the blood was when the dog began to pant in the experiment above referred to. The experiment shows that exposure of a small part of the body, mouth, neck, and fore limbs, to this not very high temperature is sufficient to

produce quickened breathing and even panting, although the animal's temperature is not raised. Human experience agrees with this ; if in the effort of getting into perspiration by means of a hot-air bath one keeps the head under the sheet and thus breathes air of about the body temperature one finds the respirations similarly increase in frequency.

In the former paper it was shown, that the temperatures there employed (41·3) did not produce the panting when the cord had been cut, and it was left for further investigation whether higher blood temperatures might produce such an effect by action on the centres directly. The setting in of panting in Exp. 1 when the dog had reached the temperature of over 42 might be adduced to support the view, that the heat in conditions like the above acts centrally, the cord having been cut. But the foregoing remarks show that such a conclusion would not be justified, as the peripheral influences from mouth and head are not excluded ; nor were those from the lung nerves. I cannot see how to throw out these peripheral influences altogether, and the question, possibly, must remain an open one, although there cannot be adduced any fact showing a direct action of heat on the centres.

A third experiment, see Table III., however, was devised in which peripheral influences were eliminated as much as possible.

Table III.

January 9, 1880.

No. of observation.	Time.	Temp. in apparatus.	Temp. of animal.	Rate of respiration.	
1	10.30		36·7		Cord and pneumogastrics are cut.
2	11.40	14	32	7	
3	12.43	13	30	6	
4	12.52	40	30	6	Placed in apparatus ; head and fore limbs free.
5	1.35	57	31	6	
6	2.53	50	34	9	
7	3.03	50	36	9	
8	3.10	53	37	10	
9	3.24	60	38	10	Ice in cloths placed around head.
10	3.29	60	38·5	9	

*2—2

No. of observation.	Time.	Temp. in apparatus.	Temp. of animal.	Rate of respiration.	
11	3.45	65	38·9	10	
12	4.14	67	40	8	
13	17	67	40·5	10	
14	24	63	41	13	
15	30	60	41·3	12	Artificial respiration necessary.
16	31				Respiration shallow and weak.
17	33	59			Artificial respiration necessary.
18	34		41·4	12	Artificial respiration necessary.
19	36	59			Respirations shallow.
20	40		41·7	12	Muscles twitching.
21	45			18	
22	49	58	42	20	Efforts at respirations rather than respirations.
23	50		42	16	
24	55		42		Artificial respiration.
25	57	58	42		Dog died.

Table III. then shows that when cord and pneumogastrics are cut the increase in the number of respirations is very low indeed. This certainly does not look as if the hot blood had the power to directly diminish resistance and increase the irritability of the respiratory centre. It is not without interest to observe how the panting can be produced if the cord is cut and the pneumogastrics preserved—in that case, however, the temperature must be, raised considerably—and how it can likewise be produced when the pneumogastrics are cut and the cord left intact, in that case the temperature need hardly be raised at all. But when both cord and pneumogastrics are cut panting is not seen, excepting under certain artificial conditions.

The next question, then, would be how much is due to the peripheral stimulation of the vagus-endings in the lungs by the increased temperature, and do they act just like the nerves of the skin? Are they sensitive to warmth?

Exp. 4, Table IV., may help to answer this question.

Table IV.

March 4th, 1880.

No. of observation.	Time.	Temp. in apparatus.	Temp. of animal.	Rate of respiration.	
1	7.35		39·2	36	The temp. in this exp. from No. 9 onwards refers to the heated air in the can. The evening was very warm and close.
2	45		39·2	45	
3	55	34	39·1	70	
4	8.00		39·2	184	
5	15				Cord cut.
6	23		38·8	50	
7	28		38·8	47	
8	40		38·5	38	Dog's trachea-tube connected with a large tin containing water at elevated temp. and Ba. (O.H.)₂.
9	55	48	38·4	36	
10	9.05	50	38·4	32	
11	23	53	38·3	29	
12	38	53	38·3	28	
13	43				Placed in warm apparatus.
14	10.06	59	38·8	32	
15	12	61	39·2	52	
16	15	58	39·5	60	
17	20	60	39·9	90	
18	25		40·4	160	
19	28	60	40·5	176	Pneumogastrics cut.
20	32	60	40·9	232	
					March 5th.
21	8.40		34·5	21	
22	45	41	.		Placed in apparatus.
23	9.08	52	35	16	
24	37	63	37	14	
25	48	54	38	18	
26	10.01	55	39·2	19	
27	06	49	40	20	
28	20	50	41	8	
29	30	51	41	14	
30	40	50	41·8	10	
31	48	50	42	44	Artificial respirations for two minutes.
32	55	50	42·5	52	
33	11.00	51	42·6	52	
34	05	52	43·2	36	
35	15		43·6	156	

We can gather, then, from Table IV. that giving the animal warm and moist air to breathe did not seem to have any effect on the peripheral vagus fibres, the animal was not made to pant thus; and, again, cutting he nerves did not stop the panting after it had once been set up. The same observation was made on a dog in which the cord was intact, the animal breathing hot air. The respirations were not permanently diminished by cutting the vagi.

But why did the dog not pant the next day after reaching a temperature of 41 ? Or why not in Table III. ?

I may add here that the dog would not have reached before dying the high temperatures which it did in Table IV. if artificial respiration had not assisted him ; and, further, the observation has repeatedly been made, that the respirations go up in frequency during artificial respiration and remains high a little time afterwards.

Regarding the depth of the respirations, I cannot support the statement that they grow deeper. Tracings which I have taken show that they grow more shallow, as it also appears to ordinary observation. Accidentally I found out, I think, how Fick's statements, that they grow deeper, came to be made. In an experiment which I made the board on which the dog rested got a little too hot accidentally, and then the respiratory movements grew deeper. As soon as the animal was protected from pain they went back to their normal character, showing more limited excursions than the respiration at the normal temperature.

THE INFLUENCE OF QUININE UPON THE REFLEX-EXCITABILITY OF THE SPINAL CORD. By Wм. T. SEDGWICK, Ph. B., *Fellow of the Johns Hopkins University, Baltimore, U.S.A.*

It is the object of this paper to describe a series of experiments which seems to indicate a different explanation from that commonly accepted for the influence of quinine upon the reflex-excitability of the spinal cord. A knowledge of the real action of this drug is particularly desirable at the present time, because Setschenow's theory of special reflex-inhibition centres has been so often and so successfully attacked that the arguments drawn from the marked effect of quinine upon reflex-irritability are to-day, perhaps, among the best reasons for retaining it.

The theory of Setschenow[1] was originally offered to explain the great loss of reflex-irritability which is the uniform result of stimulating with sodium chloride the optic lobes or optic thalami of the frog's brain. It has also been looked upon with favour as accounting most easily for that singular rise in reflex-irritability which follows division of the medulla in the normal frog.

Herzen[2] weakened the argument for the existence of these centres by showing that a depression of irritability was not limited to stimulation of the optic lobes and thalami, but might be induced by stimulation of the cord itself. Goltz[3], since that time, has removed the necessity of retaining the theory of Setschenow to explain the increased irritability of the normal frog after division of the medulla by bringing forward his theory of simultaneous stimulation. Besides these investigators, Freusberg[4] and others have tested and finally abandoned the doctrine of special reflex-inhibition centres. Nevertheless, this doctrine still offers the readiest explanation of numerous phenomena in physio-

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[1] *Ueber die Hemmungsmechanismen für die Reflexthätigkeit des Rückenmarks*, 1863. Setschenow und Paschutin, *Neue Versuche*, 1865.

[2] *Exp. sur les Centres modérateurs de l'action réflexe*, 1864.

[3] *Beiträge zur Lehre von den Functionen der Nervencentren des Frosches*, s. 39, u.s.w. Berlin, 1869.

[4] Pflüger's *Archiv*, x. (1875). 174.

logy, one of which is the remarkable loss of reflex-excitability following
the administration of a small dose of quinine to a normal frog.

Except Meihuizen[1], whose work I shall review further on, no one,
so far as I know, has offered any other explanation of the action of this
alkaloid than that it stimulates the so-called centres of Setschenow.
Chapéron[2] suggested that it probably acted in this way, and believed
that he had proved it beyond a doubt by experiments which are so simple
and yet seemingly so conclusive that they have been widely adopted for
demonstration purposes.

Thus, if a small dose of some salt of quinine be thrown under the
skin of a normal frog, or one from which only the cerebral hemispheres
have been removed (and which we may conveniently call an "optic-
lobe" frog), a great loss of reflex-excitability occurs. If now we divide
the medulla, the excitability returns quickly to the normal. Conversely,
if the medulla is divided before the dose is given, no loss of irritability
can be detected.

It has also been found that, with large doses, the reflex-irritability
may be depressed after a time even in the pithed frog.

Having thus rapidly sketched our present knowledge of the working
of this drug, I shall next describe my methods of experimenting; then
will follow a discussion of my work, a few words concerning the case
of sodium chloride, and, finally, the application of my results to the
general theories of reflex-inhibition.

Methods of Experimenting.

In spite of the objections which have been made to it, Türck's
method for measuring the reflex-irritability was used throughout.
Cyon's objection that, what one measures in such cases is not reflex-
excitability, but only the duration of the reflex-time, seems groundless.
We know that stimuli in the nervous central organs are cumulative, and
if a longer time elapses between the application of acid to the skin
and the occurrence of a reflex movement, this can only mean that the
stimulation had to attain a greater height before it gave rise to an
efferent discharge. The objection has also been raised that, sooner or
later, the acid employed must act harmfully upon the bit of skin to
which it is again and again applied. It is claimed, too, that the suspen-

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[1] Pflüger's *Archir,* VII., 216.
[2] Pflüger's *Archir,* II., 293.

sion of the frog puts him in such an abnormal position that the results
obtained are not trustworthy. These objections, and many more, dis-
appear in the light of experience gained by observations made with a
careful attention to details.

I have worked only upon frogs. Except in a few cases they were
hung up by a large pin (passing through the head between the nares),
from either end of a horizontal wooden bar. This bar was supported by
having its middle portion nailed to a tall block, so that no other part of
the frog's body was in contact with any solid object. A reservoir of
water above communicating with a flexible rubber tube closed by a pinch-
cock gave abundant and ready means for washing the frogs and keeping
them in good order. They were constantly watched, and frequently
bathed by immersion in a basin of water lifted from below. This basin
of water is also a quick means of removing the acid after the reflex
movement has occurred. Draughts of air were found very irritating, and
were, therefore, avoided.

Dilute sulphuric acid was employed, and was made by diluting to a
litre two c. c. of commercial "pure sulphuric acid." It had a quite
distinctly acid taste. The time was marked off by a metronome, beating
one hundred strokes a minute. At first the reflex-irritability was esti-
mated every ten minutes; however, if the conditions are good, five
minutes is a sufficient interval, and my later observations were made
five minutes apart. No comparative experiments were attempted until the
record of several consecutive reflexes showed only such variations as
would fall within the limits of observation errors.

Perhaps the greatest difficulty met with in using the method of
Türck is to be sure that the toe of the frog dips into the acid equally far
every time the reflexes are determined. Carelessness in this respect may
produce great variations in a record, and for this reason Meihuizen's
plan of holding the frog in the hand is objectionable. Again, the acid
must be removed with all possible speed after the reflex movement has
taken place.

As I employed it, Türck's method gave satisfactory results; for
frogs could usually be kept in good order as long as was needful. A
test experiment, in which two frogs had their medullas divided, and
soon after were hung up as I have said, showed a record of reflexes
which hardly varied for six hours. The irritability was taken every five
minutes. So that they were suspended in an abnormal position for nearly
six hours; they had sixty-six applications of dilute acid to the same bit of
skin; these sixty-six stimuli set up as many reflex movements; yet at

the end of the trial the reflex excitability was precisely the same as at the beginning, and observations ceased only from my own weariness. In the face of such experiments it seems absurd to claim that, under proper precautions, repeated applications of acid of the strength indicated, or repeated demands upon the spinal cord, will lead to serious errors.

It is a matter worthy of close attention, especially in view of the results which I have reached, to consider the form in which quinine shall be given. As Hermann[1] points out, the use of acid to dissolve the sulphate is not to be recommended ; for the acid may set up stimuli which will depress the reflexes like other stimulation of sensory nerves. Yet, if sulphate of quinine is to be used at all, acid must be added, for it is little soluble in pure water. For these reasons it seems best to reject the sulphate, and to use the chloride, which is quite soluble in pure water, and weight for weight, contains much more quinine. There is, however, one danger in using this salt which must be borne in mind. If given in doses of a rather concentrated solution it behaves as an irritant. Commonly the drug is injected under the opaque skin of the frog's back. Thinking that less danger of losing any of the dose was incurred by putting it under the abdominal skin (as the frogs sometimes jump about, and by arching the back squeeze out a few drops), I have lately thrown the drug in at a small incision on the abdominal skin near one of the arms. I have noticed, with some surprise, that, after a time, there often appears a large congested area just over the part where most of the solution is lying. If the aqueous solution of quinine chloride may act in this way it suggests that it should always be dilute ; for if irritating it is quite as objectionable as the acid solution of the sulphate. For ordinary work I have used a freshly made solution, ·06 grams of quinine chloride in 10 c. c. of distilled water. This does not appear to irritate ; and using ½ c. c., which is a convenient quantity, a dose of ·003 grams is given. The solution of atropia which I employed had ·005 grams of the sulphate dissolved in 10 c. c. of water ; this gave for each dose of ½ c. c. only ·00025 grams, yet this minute quantity proved ample.

Quinine Salts.

As has been said above, so far as I know, the only attempt to explain the action of these salts on any other theory than that they stimulate the so-called centres of Setschenow has been made by Meihuizen[2],

[1] *Lehrbuch der Experimentellen Toxicologie*, s. 366. Berlin, 1874.
[2] Pflüger's *Archir*, VII., 216.

and by him only indirectly. He worked only with frogs whose medullas had been divided, so that these particular centres were out of the question. Still, he advanced a theory for the action of the chloride of quinine on such frogs which, if true there, might also be true perhaps in the entire or optic-lobe frog. It was thought best, therefore, to test his theory. Meihuizen found—and I agree with him in this—that although in the frog whose medulla has been divided small doses of quinine do not seem to affect either the heart-beat or the reflex-excitability, large doses do, on the contrary, affect both. They slow the heart-beat and depress the reflex-excitability.

In his other work I have not been able to confirm Meihuizen's results. Under large doses of quinine I have repeatedly seen the reflex-excitability grow feebler and feebler, till it finally disappeared altogether. In such cases I have almost invariably found the heart still beating, though the circulation in the web-vessels was usually stopped. Meihuizen, on the other hand, finds no loss of reflex-excitability until the heart has wholly stopped beating; then, he says, the reflexes disappear in from fifteen to thirty minutes, or often even sooner—that is to say, a great loss of reflex-excitability never *precedes* a cessation of the heart-beat. On this observation he builds his theory, which is, that in frogs with divided medullas quinine depresses the reflexes by producing grave disturbances in the circulation. I can only reconcile my own results with his by supposing that the exposure of the heart which he resorted to in some way causes it to stop sooner than it otherwise would. Different as the case is from that of the ordinary frog supposed to have inhibition-centres, it might be that in the latter the circulation was affected even when no obvious change was seen; and, as a consequence, by virtue of these centres, quickly depressed the reflexes of the spinal cord. Experiments were therefore begun both with quinine and with sodium chloride, in order to settle the point upon frogs having the so-called centres of Setschenow. The heart having been exposed in an optic-lobe frog, and a crystal of sodium chloride laid on the cut ends of the thalami, no change in the heart-beat is seen for a short time; very soon, however, the heart beats slower, becomes dilated, and stops in diastole, with all the phenomena of vagus-inhibition. Almost at the same time convulsions usually begin, and when they are over the heart is found beating again. If the vagi are cut beforehand the heart cannot be stopped in this way; and so, too, if a minute dose of atropia is given before beginning the experiment it always fails; hence we are probably safe in concluding that the phe-

nomenon is due to vagus-inhibition of the heart-beat, brought about by stimulation of the thalami with sodium chloride. This is a fact of some interest, perhaps. So far, support seemed likely to be given to the theory of Meihuizen. Accordingly, the work upon quinine chloride was begun with special interest, for I did not then know that quinine forbids vagus-inhibition. I soon found, however, that in the entire or optic-lobe frog the heart was not stopped in the same way by small doses of quinine. Moreover, a dose large enough to slow the heart-beat, or to stop it, continues its effect even after that organ has been separated from its extrinsic nerves. Clearly, the cases of quinine and sodium chloride are very unlike, so far as the heart is concerned. I next proceeded to estimate directly the influence upon the reflexes of profound disturbance of the circulation. The reflex-time in frogs with divided medullas having been carefully recorded and found fairly constant, the heart was exposed, and a ligature passed tightly around it, so that all circulation stopped at once. This experiment seemed to show that in no case did the reflex-time change much within half an hour ; and this, it will be remembered, was the extreme period during which, according to Meihuizen, the reflexes lingered after total stoppage of the heart-beat by quinine. Table I. records some experiments made in April on frogs in good order, and under the same conditions. All were tested at the same time, the animals being hung up side by side, and observed one after the other at equal intervals. When it is recollected that, although the incision to expose the heart does not perceptibly affect the reflex-time, ligaturing-off the heart is a more profound operation, the moderate variations which the records indicate may perhaps be well accounted for. On the average, about forty minutes elapsed before the reflex-irritability suffered any great change ; even then the reflexes seemed to fail rather from stiffening of the muscles than from any change in the nervous elements. From the fact which these experiments seem to prove, that a total stoppage of the circulation has less rapid effect upon the reflexes than even large doses of quinine, we must conclude that quinine does not act primarily upon reflex-excitability by diminishing the blood-flow.

EXPERIMENT 1.

The observations were made ten minutes apart. Frogs A, B, C, D, E, F, with heart ligatured, show the effect of a total stoppage of circulation upon reflex-irritability : their medullas had been divided one

hour before experiments began. E was an optic-lobe frog whose hemispheres had been removed several hours before. Frogs G, H, and Z give an opportunity for comparing the effect of large doses of quinine with a complete stoppage of circulation. Z had a dilute, and G and H had a concentrated, dose of quinine.

Table I.

No. of observation.	Time.	Frog A.	Frog B.	Frog C.	Frog D.	Frog E.	Frog F.	Frog G.	Frog H.	Frog Z.	REMARKS.
1	10.40	10	8	3	8	8	5	6	6	5	
2	10.50	5	3	3	4	9	6	6	4	6	"50 + " means that
3	11.00	6	4	2	8	8	5	5	4	5	the metronome beat
4	11.10	5	5	3	4	9	5	5	4	6	over 50 times, and no reflex movement was
5	11.20	Heart tied.	Heart tied.	Heart tied.	Heart tied.	Heart tied.	Heart tied	+ Q	+ Q	+ Q	seen.
6	11.30	8	10	3	6	10	5	10	9	4	The ligatures were
7	11.40	7	7	4	7	17	5	13	9	5	put on just as soon as the observations
8	11.50	7	11	4	7	15	6	50 +	50 +	6	given under 11.10
9	12.00	9	7	6	7	26	8	50 +	50 +	7	were over, although the Table shows 11.20
10	12.10	19	8	9?	50 +	50 +	50 +	etc.	etc.	9	as the real time. The
11	12.20	50 +	10	50 +	50 +	50 +	50 +			50 +	average time which elapsed between the
12	12.30	50 +	11	50 +	etc.	etc.	etc.			50 +	application of the ligature and the loss
13	12.40	etc.	12?	etc.						etc.	of all reflex indicated
14	12.50		50 +								by the second 50 +
15	1.00		50 +								was not less than 45
16	1.10		etc.								minutes.

Having in mind the remarkable inhibition of the heart-beat by sodium chloride applied to the mid-brain of the frog, which seems to point clearly to a distinct efferent impulse proceeding from the stimulated part, and recollecting the various phenomena of simultaneous stimulation, such as the diminished irritability in one leg when the sciatic nerve of the other is stimulated by a strong electric current, it is not difficult to suppose that all the reflex-inhibitions produced by applying sodium chloride to the nervous apparatus of the frog are special cases of simultaneous stimulation.

Turning next to quinine, and attempting to apply, in this case, the same theory, our attention is at once drawn to the important fact that quinine has a decided effect upon the heart itself. Something is certainly going on here, for the heart beats slowly under a moderate dose and ceases to beat altogether under a large one.

If counter nervous stimulation occurs in this organ it must be

through the vagus nerve. If this acts as the afferent nerve, whose stimulation is to depress reflex-excitability, then division of the medulla below the nerve must forbid that depression, as it does. By reviewing the subject and by this train of thought I was led to believe that such a theory would account well for the facts and do away with the necessity for supposing the existence, in this case, of special inhibition centres; it would be this: quinine salts acting upon the nervous network of the heart, stimulate the vagus nerve, and so depress general reflex-irritability in a way similar to that in which electrical stimulation of one sciatic nerve may depress the reflexes in the other leg.

This theory accounts well for facts which have long been thoroughly established, and, if true, need meet with little objection, for its depressing effects upon the reflex-excitability of the cord are only simplified and placed alongside of many other cases of simultaneous stimulation which are unquestioned. The return of that excitability after division of the medulla is accounted for, since the source of the depression — the stimulated nerve—is no longer connected with the cord; and, conversely, if the medulla is divided beforehand no depression can occur for the same reason. Moreover, the effects of small and large doses upon frogs with divided medulla should be, as they are, totally unlike the effects of the same doses upon normal or optic-lobe frogs.

If my theory is true, section of the vagus nerves ought to be, so far as the reflexes are concerned, equivalent to dividing the medulla. Accordingly I divided both vagi close to the medulla, but the results were not constant. Owing to paralysis of the laryngeal muscles the frogs no longer breathed normally and always bore the marks of a too severe operation. No absolutely contradictory results were obtained; still sometimes, after quinine-giving, the reflexes fell, but as it seemed rather from general exhaustion of the animal, and in others the reflexes continued as if no quinine had been given. Division of the visceral branches of the vagi below the origin of the laryngeal was a less severe operation, and was correspondingly more successful. A great trouble in this mode of experimenting is that a very considerable number of the frogs after the operation swell up enormously and utterly fail to expire. The phenomenon is described by Heinemann, and frogs which show it are no longer available for experiments upon reflex-irritability. Then, too, even of those which do not seem affected in that way, it may be true that there is something going on which, while it is not conspicuous, may, notwithstanding, affect the reflexes. Those frogs which showed no signs of Heinemann's phenomenon gave very fair results.

Exp. 2, see Table II., records some of these results and affords an opportunity for comparing them with the effects of similar doses upon the normal frog. It should not be forgotten that an animal which has undergone a severe operation cannot be expected to retain irritability so long as an animal unoperated upon.

EXPERIMENT 2.

The observations took place five minutes apart. For convenience they are arranged as if they were made simultaneously. They represent some of the best cases for the theory.

A, B, C, D had had the visceral branches of their vagi divided below the origin of the laryngeal several hours before.

E was an optic-lobe frog. F, G, and H had undergone no operation.

The experiments occurred in April and May, 1880, and the weather was favourable for the work. Frogs were chosen with special reference to the apparent absence of Heinemann's phenomenon.

The results thus far obtained, though very encouraging, were not perfectly satisfactory. It was therefore decided to make use of atropia, in the hope that, since it is believed to paralyse the inhibitory vagus-endings in the heart, it might also paralyse the ends of the afferent fibres, and so prevent the action of quinine, which, the theory supposes, stimulates those endings. This test proved perfectly satisfactory. Table III. shows several cases, which may be compared with others taken at the same time when no atropia was given. They are representative examples. The dose of sulphate of atropia (·00025 grams) is very small, but after it has been given the usual amount of quinine seems to have no effect at all.

It will be remarked that the small quantity of atropia does not itself affect the reflexes. This I have proved by separate experiments, see Exp. 3, Table III.

Table II.

No. of observation.	Time.	Frog A.	Frog B.	Frog C.	Frog D.	Frog E.	Frog F.	Frog G.	Frog H.	REMARKS.
		With vagi divided.								
1	3.10	10	6	8	10	10	10	11	6	
2	3.15	9	7	6	11	9	12	10	6	
3	3.20	8	6	7	9	8	11	11	5	
4	3.25	8	5	7	5	7	9	9	6	
5	3.30	7	6	6	4	7	10	10	5	
6	3.35	8	6	7	4	8	11	9	5	For the signi-
7	3.40	↓Q	↓Q	↓Q	↓Q	↓Q	↓Q	↓Q	↓Q	ficance of "50+"
8	3.45	22	6	—	4	11	24	—	11	see Table I.
9	3.50	9	7	8	3	11	13	10	10	
10	3.55	8	6	7	3	9	16	9	11	
11	4.00	11	6	9	4	7	15	13	12	
12	4.05	10	6	9	3	8	25	20	10	
13	4.10	12	9	9	4	15	40	16	12	"M. d." means
14	4.15	10	11	11	More. ↓Q	14	M. d.	17	18	"medulla di-
15	4.20	8	11	—	5	14	—	23	23	vided."
16	4.25	9	9	—	5	28	—	—	46	
17	4.30	—	10	—	5	50+	—	—	etc.	
18	4.35	—	14	—	4	50+	20	—		
19	4.40	—	12	—	4	M. d.	26	—		The dash is
20	4.45	—	12		4	—	15	—		used instead of
21	4.50	—	12		5	21	18	—		the words, "No
22	4.55		12		More. ↓Q	13	13			observation."
23	5.00		29		8	18	13			
24	5.05		30		10	13	—			
25	5.10		M. d.		12	—	—			
26	5.15		—		11	—	—			D had, in all,
27	5.20		—		11	—	—			three large doses
28	5.25		No		10		—			of quinine.
29	5.30		reflex.		13		—			
30	5.35		—		37					
31	5.40				50+					
32	5.45				50+					
33	5.50				Dead.					

If proof for my theory depended solely upon the action of atropia, it might properly be argued that we know too little of the action of this drug to base upon its effects any explanation of the working of quinine ; but when taken in connection with the effects produced by vagus-section, it becomes a valuable ally for the theory. Owing to the sudden advent of warm weather I have made but a single experiment to see if atropin was a general paralyser of inhibitory fibres. An optic-lobe frog, to which a large dose of atropia had been given, showed the ordinary loss of reflex-irritability when his lobes were stimulated with salt. Moreover, it is hardly possible that the small dose which I have used could prevent general reflex-inhibition.

These experiments seem to me to show that quinine salts, when given to the normal or optic-lobe frog in small doses, depress the reflex-excitability by stimulating the vagus nerve through its endings in the heart. It is not unlikely that the pulmonary and gastric endings may also be influenced, but I have no proof of their action.

If my work shall be confirmed, it must be admitted that in the frog with divided medulla we have a different problem to solve. Small doses are here ineffectual ; and when we recollect that quinine is a proto-plasmic poison, and in large or concentrated doses may become an irritant, several possibilities arise. Quinine may poison the cord directly, or have some other equally obscure action ; but from some experiments which I have begun but have not yet completed, it is possible that the depression in these cases is due to intense simultaneous stimulation ; the irritating quinine solution being a stimulus comparable to the electric stimulus applied to a sciatic nerve, and, like that, affecting materially the general reflex-excitability. That it acts more feebly in case the brain and great nerve-centres are gone is to be expected ; it has less to work with and upon.

Sodium Chloride.

My work upon the behaviour of this substance has not perhaps gone beyond that of other observers. Their accepted results I have been able to confirm in most cases. Herzen's observation that stimulation of the cord could cause a depression of excitability I have fully confirmed by dividing the medulla, estimating the reflex-time before and after placing salt upon the section. On cutting across the cord again below

EXPERIMENT 3.

Observations occurred five minutes apart. Frogs A, B, C, D, E show that atropin does not in such doses affect the reflexes; also that after atropin-giving quinine is ineffectual. F, G, H, I show the effect of the same doses of quinine when no atropin has been given. In no case after atropin-giving have I seen quinine have its ordinary effect.

Table III.

No. of observation.	Time.	Frog A.	Frog B.	Frog C.	Frog D.	Frog E.	Frog F.	Frog G.	Frog H.	Frog I.	Remarks.
		Quinine preceded by atropin.					No atropin beforehand.				
1	9.25	9	7	7	—	8	7	—	7	10	
2	9.30	9	4	5	9	9	8	9	9	9	
3	9.35	10	4	4	9	9	7	8	7	10	
4	9.40	7	3	3	8	10	9	8	8	10	
5	9.45	7	3	4	8	9	9	7	9	10	
6	9.50	A SO	A SO₄	A SO₄	A SO₄	A SO	·003 Q Cl.	·003 Q Cl.	·006 Q Cl.	·003 Q Cl.	For the significance of "50+" and the dash, see Tables I. and II.
7	9.55	8	8	6	9	10	22	14	35	17	
8	10.00	8	4	5	7	9	20	13	48	16	
9	10.05	8	4	4	8	10	18	14	50+	14	
10	10.10	8	5	3	6	10	24	16	50+	21	
11	10.15	8	4	4	7	9	25	15	etc.	16	Owing to a mistake H had a double dose.
12	10.20	·003 Q Cl.	·006 Q Cl.	·003 Q Cl.	·003 Q Cl.	·003 Q Cl.	27	20		18	
13	10.25	9	4	5	—	9	37	17		16	
14	10.30	8	7	5	7	10	50+	50+	A SO₄		Frog F shows about the average effect of this dose (·003) of quinine chloride as I have frequently observed it.
15	10.35	5	8	6	9	9	50+	50+	28		
16	10.40	6	7	4	8	10	etc.	etc.	13		
17	10.45	7	9	5	8	9			25		
18	10.50	8	10	6	8	8			25		
19	10.55	9	10	7	12	·003 more.			A SO₄		
20	11.00	9	10	6	11	8			24		
21	11.05	9	6	5	—	9			50+		Frog I's record shows that this dose (·00025) of atropin sulphate did not restore the reflex irritability.
22	11.10	9	7	4	8	9			50+		
23	11.15	8	7	5	10	9			etc.		
24	11.20	7	etc.	4	7	—					
25	11.25	8		etc.	6	10					
26	11.30	8			6	11					
27	11.35	7			5	12					
28	11.40	6			etc.	10					
29	11.45	7				9					
30	11.50	etc.				etc.					

this point the reflexes may often be restored; they may then be again depressed by salt and restored by section.

I wish merely to call attention to the evidences of simultaneous stimulation in the case of sodium chloride, as bearing upon theory. These evidences are, first, the fact that the heart may be stopped by applying salt to the thalami; second, that at about the same time convulsions occur; and, third, these are not due to stimulation of the so-called "convulsive centre," since they occur almost as well if the salt is laid upon the cut cord from which the medulla has been removed. These facts, if they show anything, show that the salt may act as a direct stimulus of considerable power.

THEORETICAL CONSIDERATIONS.

I have not overlooked the difficulties which seem to arise from the strange behaviour of atropia towards quinine depression of reflex-excitability. It is not easy to understand how two drugs which have, apparently, the same effect upon the inhibitory function of the vagus shall nevertheless act precisely unlike upon the vagus nerve in respect to reflex depression. At first sight we can only escape by saying that it (quinine) acts as a paralyser of inhibitory endings and as an excitant of afferent endings of the vagus nerve, while atropin paralyses both. This hypothesis, however, assumes a distribution of function in the vagus fibres which we are hardly justified in making. In view of the discovery by Prof. H. Newell Martin[1], that special reflexes may be inhibited by the stimulation of the central ends of *efferent fibres*, we may have to change all our ideas of reflex-inhibition, and it may be that quinine merely stimulates the ends of the efferent cardio-inhibitory fibres, and these act back in the centres.

Since atropin is known to paralyse the peripheral organs of the cardio-inhibitory fibres, we would then get an explanation of the fact that after its administration small doses of quinine are without effect on the reflexes. Otherwise it would appear that we must assume that atropin paralyses also the ending of afferent vagus fibres in the heart,

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[1] Johns Hopkins, *University Circular*, May, 1880. A preliminary account of some experiments tending to prove the existence of a new function in the anterior roots of the spinal nerves.

which are stimulated in the organ under the influence of quinine and depress the reflexes.

The slowing of the heart under quinine, and at the same time the loss of cardiac inhibition on direct vagus stimulation, show that the cardiac action of the drug still needs much more investigation.

If the statement which was made at the outset, that the theory of Setschenow is better sustained by quinine than almost anything else, is true, then it must be granted that that theory now rests on a weak support. If my results secure confirmation, quinine does not depress the reflexes by the mediation of any special inhibitory centres. Moreover, it seems to me that all the phenomena found in using common salt to demonstrate the existence of these centres may be better explained by looking at them as particular cases of simultaneous stimulation, comparable to the general inhibition of reflexes accompanying the powerful stimulation of a sensory nerve.

Sodium chloride, although its first cause, has for some years been a stumbling-block in the way of the theory of Setschenow, while quinine has been one of its most important supports. Goltz's theory, on the contrary, has been made more probable by the action of salt, and has hardly accounted for the effect of quinine. It will be seen that the results of my work support Goltz and render highly improbable the theory of Setschenow.   .

The general results of this paper may be stated thus :—

1.   Quinine salts in small doses seem to depress the reflex-excitability of the cord by stimulation of the vagus nerve ; mainly through its endings in the heart.

2.   This places the quinine action alongside other stimuli of sensory nerves, and explains it action by saying that it is a special case of reflex depression by simultaneous stimulation.

3.   Goltz's theory is supported, and that of Setschenow much weakened by these phenomena.

4.   Reflex depression under quinine salts, in the pithed frog, is a case wholly different from the same depression in the entire frog. Larger doses are required, and the drug possibly acts as a direct poison on the cord.

It is not unlikely that other drugs may act like quinine upon the

reflexes. I propose to continue my work and shall especially examine digitalis, and others which act upon the heart.

THE EARLY DEVELOPMENT OF THE WOLFF-
IAN BODY IN AMBLYSTOMA PUNCTATUM.

By SAMUEL F. CLARKE, Ph. D., *Late Fellow and Assistant in Biology, Johns Hopkins University.* With Plates I, II and III.

THE first indication of the urinogenital system in Amblystoma is found at the period of development represented in Figure 1. At this stage, as seen in cross sections, Figures 4 N to 13 N, the mesoderm extends entirely around the body forming a two-celled lamella. In the region which is to become the intermediate cell mass, both layers of mesoderm are much enlarged, see Figures 4 N to 12 N. This enlargement of the mesoderm is produced by a growth of and not a multiplication of the cells, as is seen in the Figures of series "N." This beginning of the Wolffian blastema was found extending through a few sections only in a consecutive series. In the next later stage from which a series was obtained, Figure 2, the somatopleure cells of the blastema have become very much larger than those of the splanchnopleure; the former divide transversely and then become differentiated from the rest of the mesoderm by a definite outline. This blastema now consists of a solid mass or rod of cells lying just ventral to the lateral plates, bounded on the inside by the splanchnopleure, on the outside by the epiderm and formed from the outer layer of mesoderm. At its anterior end through six or seven sections it is of considerable size, then it suddenly becomes much smaller and continues without change through ten or twelve sections farther backward. In the next succeeding series of sections, taken from a specimen represented in Figure 40, one finds that the body cavity is beginning to be formed and the Wolffian blastema is seen to be entirely in the somatopleure; no anterior opening has yet been formed in the segmental duct, as Balfour has called this structure in the Elasmobranchs, as is demonstrated by the first section. The next two sections show that a lumen is being formed within the previously solid rod, while the three sections following these two indicate a partial differentiation of the blastema into a dorsal and ventral part. After one or two sections more, the dorsal portion terminates and the ventral part continues posteriorly as a solid rod.

4   ·   39

The next or fourth series are from an embryo represented in Figure 41 Y. In this stage one finds from the sections that the dorsal duct now opens anteriorly into the body-cavity ; the split has worked its way forward to the anterior end of the blastema, separating the anterior end into two quite separate parts or ducts, each with a lumen, but the ventral one ends blindly while the dorsal one communicates with the body-cavity. Below the ventral duct is a small solid rod of cells which was, I believe, not formed from the blastema. In section number 37 Y of this series the dorsal and ventral ducts have united into one which possesses a single large lumen. The next succeeding section shows this single duct opening into the body-cavity.

The Wolffian body then, arises from the outer layer of the mesoderm as a solid rod of cells, and is at first largest anteriorly ; a split then occurs in the larger portion which begins at the posterior end of the smaller part and travels anteriorly, and at this time a lumen has appeared in the anterior end of the blastema ; finally, the split reaches the anterior end thus dividing that portion into two ducts ; the lumen is extending itself backward, a small rod of cells has been formed below the anterior end of the ventral duct, the dorsal and ventral ducts are united at one point, and a second opening into the body-cavity from the dorsal duct has been made. This method of development seems to be quite different from that in any allied forms in which the development has been worked out. As it is most like that of the Elasmobranchs, I will add a brief account of the development of the urinogenital system in the latter group as given by Balfour. It first makes its appearance as a solid knob of cells springing from the intermediate cell mass. From this knob a solid column of cells grows backwards to the level of the anus. The knob then acquires an opening into the body-cavity which is continuous with a lumen that makes its appearance in the rod of cells. Solid outgrowths of the intermediate cell mass then appear which soon become hollow and open into the body-cavity. Their blind ends curl obliquely backwards and open into the segmental duct. After all this has taken place the segmental duct splits longitudinally into two ducts in the female, and into one duct and parts of another in the male.

In comparing this with Amblystoma, one notices that the origin of the primitive rod of cells is very similar in both, they agree

in the anterior opening into the body-cavity and in the lumen appearing anteriorly and working its way backward.   Beyond these points they are unlike.   The splitting of the segmental duct in Amblystoma takes place at a much earlier period and proceeds in a different way.   The second opening into the body-cavity is also peculiar to Amblystoma as is the small rod of cells lying ventral to the two tubes which are derived from the blastema.   It is possible, however, that this small rod is not a part of the urino-genital system; and this second opening into the body-cavity is probably the beginning of the first segmental tube.

It is a matter of great regret to me that I have not sufficiently complete results to allow of any theoretical considerations, and I have concluded to publish this short descriptive paper because there is enough to show that the method of development of the Urinogenital system in Amblystoma is quite different from that of allied forms, and indicates a promising field of work, if the sections can be obtained.   I have worked many months to obtain the few results here recorded, so difficult is it to obtain workable material. Many thousands of sections have been prepared and mounted, nearly all of which from one cause or another are valueless; many are utterly worthless, while a large number, though partly good, are not reliable.   I have had the best results with Picric acid specimens, and find that they work better a few days after they have been transferred to absolute Alcohol, than when longer kept.

EXPLANATION OF PLATES.

The figures are numbered from 1 to 41 and the different series of sections are indicated by letters annexed to the numbers of the figures.

All of the figures were outlined with the aid of the camera lucida.

PLATE I.

FIGURE 1.—A side view of the specimen from which the series of sections marked "*N*" were obtained.   *nc,* neural canal; *e,* eye; *t,* throat; *a,* future position of cloaca.   Magnified six diameters.

FIGURE 2.—A side view of the specimen from which the series of sections marked "*P*" were made.   *'e,* eye; *mb,* mid-

FIGURE 2.—*Continued.*

       brain ; *bn,* branchial lobe ; *ba,* brachial lobe, from which the anterior limb is developed ; *pr,* protovertebrae. Enlarged six diameters.

FIGURE 3.—A diagrammatic figure of the developing Wolffian body of Amblystoma made from series "*Y.*" *pp,* bodycavity ; *b*1, the dorsal duct, opening anteriorly into the body-cavity ; *x,* its second opening into the bodycavity ; *b*2, the ventral duct which unites with the dorsal duct just in front of the second opening of the latter ; *b*3, the small rod of cells which appears just beneath the ventral of the two large ducts.

FIGURE 4 *N.*—A cross-section through the body at the anterior end of that enlarged portion of the mesoderm from which the Wolffian blastema is formed. The hypoblast cells are very large and filled with very coarsely granular protoplasm ; *ac,* the alimentary canal ; *nt,* the notochord which appears to be formed from the hypoblast ; *wb,* the enlarged part of the mesoderm from which the Wolffian blastema is formed. The mesoblast at this stage extends entirely around the body, forming a twocelled lamella. *ep,* epiblast.

FIGURES 4 *N* to 8 *N,* are consecutive and show that this enlarged area of mesoderm extends through these five sections without any marked change.

PLATE II.

FIGURE 9 *N.*—This is not the next section to 8 *N,* but is next but one. The enlarged portion of mesoderm *wb,* still persists.

FIGURE 10 *N.*—This represents the next section but one to Figure 9 *N,* and shows no marked change.

FIGURE 10 *N,* to 13 *N,* are consecutive. Figure 13 indicates the posterior termination of the mesoderm marked *wb.*

FIGURE 14 *P,* to 21 *P,* are consecutive, and are taken from the specimen represented in Figure 2. The series is completed with Figures 22 and 23 on Plate III.

FIGURE 14 *P.*—A section through the anterior end of the Wolffian blastema, *wb.*

FIGURES 15 *P*, to 20 *P*, are essentially alike, showing the Wolffian blastema, *bl*, extending backward without marked change in size or form.

FIGURE 21 *P*.—In this section the blastema, *bl*, suddenly diminishes in size.

It will be seen from a comparison of sections, 14 *P* and 15 *P*, that the Wolffian blastema is found from the outer layer of cells of the mesoderm.

PLATE III.

FIGURE 22 *P*, is next but one in the series to 21 *P*. There is not much change; the intermediate cell mass with the blastema *bl*, is more distinctly separated from the protovertebræ.

FIGURE 23 *P*.—This is five sections further backward in the series than 22 *P*, and shows the blastema reduced to a small rod of cells. It occurs in one or two more sections only and then terminates.

FIGURES 26 *W* to 32 *W*, form the third series, and were made from the specimen represented in Figure 40.

FIGURE 24 *W*.—The anterior end of the blastema is shown at *bl*. The body-cavity *pp*, is beginning to be formed.

FIGURES 25 *W*, and 26 *W*.—The blastema is larger than in 24 *W*, and the body-cavity is still present.

FIGURE 27 *W*.—The blastema is here much enlarged and is being divided by a median transverse division.

FIGURE 28 *W*.—The split is here indicated also, but the upper or dorsal portion is much the largest. The body-cavity *pp*, is present but disappears in the next section.

FIGURE 29 *W*.—There are now two distinct ducts, a dorsal *bl I*, and a ventral *bl II*.

FIGURE 30 *W*.—The two ducts are still present but their lumena have disappeared.

FIGURE 31 *W*.—The dorsal duct *bl I*, here terminates while the ventral one persists.

FIGURE 32 *W*.—This is next but one in the series of sections. The now single rod of cells extends only a few sections farther.

In studying this series "*W*" it appears that the blastema in its enlarged anterior part becomes longitudinally divided by a split which starts at the posterior end of the swollen portion and travels anteriorly.

Figures 33 Y, to 39 Y, comprise the last series, and were obtained from an individual shown in Figure 41 Y.

Figure 33 Y.—A section through the anterior end of the developing Wolffian body ; *bc,* body cavity, into which opens the dorsal duct *b*1 ; *b*2, the ventral duct and *b*3, a small rod of cells which is found only in this and the following section.

Figure 34 Y.—The dorsal duct *b*1, is here distinct from the body cavity, *bc.* There is a peculiar collection of cells about the ventral duct which may be a trace of the primitive connection of the dorsal and ventral ducts, the split not being quite completed at this point. There is a small lumen in each of the two ducts. The small ventral rod of cells is also present.

Figure 35 Y.—The dorsal and ventral ducts hold the same relative positions and have the same characters.

Figure 36 Y.—The two ducts have united, forming one large duct with a large lumen.

Figure 37 Y.—The single duct here opens into the body-cavity.

Figure 38 Y.—The single duct has become a solid rod of cells, and in this condition stretches away toward the posterior end of the body.

Figure 39 Y.—This is six sections posterior to 39 Y, and beyond this the "rod" does not extend.

Figure 40.—A side view of the individual from which the series of sections marked "W," were made. *e,* eye; *ba,* branchial lobe; *bn,* branchial lobe; *pr,* protovertebræ. Enlarged six diameters.

Figure 41 Y.—A side view of the specimen from which series "Y" were obtained; *np,* nasal pit; *e,* eye; *bal,* balancer; *bn,* branchial lobe; *ba,* branchial lobe. Enlarged six diameters.

Figure 3, on Plate I, gives a diagrammatic side view of the developing Wolffian body of Amblystoma constructed from this series of sections marked "Y."

NOTES ON THE FORMATION OF DENTINE AND OF OSSEOUS TISSUE.

By CHRISTIAN SIHLER, M. D., Ph. D., *Late Fellow and Assistant in Biology, Johns Hopkins University.* With Plate V.

I. Dentine.

There are two views held regarding the formation of dentine: one supported by Wàldeyer, in Stricker's Handbook, the other by Kölliker, in his Histology. According to the former all the cells of the tooth pulp are used up in the formation and are actively engaged in the production of dentine. According to the second view the odontoblasts only are the elements whose function it is to deposit dentine. Waldeyer believes that osseous tissue and enamel develop in quite an analogous way.

I shall now bring forward the observations which I have made on the tissues coming into play in the process, and then consider which view they support.

(1). Cracking with a vice the incisor of a calf, or splitting the root with a knife, one finds that the pulp is removed from the dentine very easily indeed; great difficulty is often experienced in keeping it adherent to the dentine in order to make sections through pulp and dentine, both remaining in their natural position with reference to each other. This behavior of the pulp towards the dentine is in striking contrast to that of the pericementum towards the cementum, and seems to me to throw some light on the difference in their respective modes of growth. Although this fact is one readily observable without the microscope it seems not less important on that account.

(2). Before enumerating the facts brought out by the microscope, I shortly describe the method. The materials used were, principally, the incisors of the calf, the roots of which were split longitudinally that the staining fluid might have access to all the parts, including the dentinal canals; and care was taken to disturb the relation between the pulp and dentine as little as possible. In the staining fluid, Beale's carmine, the teeth remained, until the pulp-cells were deeply stained; after washing with acidulated glycerine, they were transferred to dilute alcohol, from this into strong alcohol, and then allowed to dry, the pulp applying itself closely—

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in some parts at least—to the dentine. Sections were then made
with a hard and fine scalpel, through all the parts, pericementum,
cementum, dentine and pulp, or through parts of these layers, as
was desired. The sections were then treated with glycerine and
acetic acid, which swells them out and brings them back to their
natural condition.

Figure 1, Plate V, shows such a section passing through the
root. *a*, pulp—near the dentine the darker red shows that the
cells there are either large or more numerous. *b*, the *pink zone*,
the newest layer of dentine which is not yet ossified. *c*, the fully
formed dentine. *f*, the pericementum. *e*, the uncalcified cemen-
tum (again a pink zone). *d*, the calcified cementum.

This drawing is intended to show only the general arrangement
of the parts, and is but little magnified.

Figure 2, Plate V, more highly magnified, shows the fully
formed dentine and the adjoining soft tissues where the growth of
the dentine must be in progress. We observe here—*a*, the calci-
fied dentine. *b*, the uncalcified dentine, (the pink zone already
mentioned). The newly laid down semi-solid material absorbs
some of the carmine, but is not stained as deeply as the protoplasm
of the cells. Next comes a layer of large and long cells, reminding
one of columnar epithelial cells, with a dark red nucleus situated
generally towards the blunt end of the cells which is directed
towards the pulp. There has never come such an odontoblast
under my notice with more than one nucleus. *d*, the pulp proper
showing oval and roundish masses of protoplasm imbedded in
formed matter of a finely fibrillated character.

(4). The elements making the pulp can readily be examined, by
teasing and scraping a pulp which, after having been removed, has
been kept in bichromate of potass solution. Figure 4, Plate V,
shows such cells. They form very irregular, branched, and varied
figures, their processes evidently running out into and continuous
with the fibrous network of the pulp. The naked eye shows, and
this must be borne in mind, that the pulp is exceedingly vascular,
and, upon teasing, larger vessels with unstriated muscular fibres,
and smaller ones richly nucleated, are observed pervading the
whole pulp.

(5). The odontoblasts in a very natural condition can be pro-
cured by scraping the freshly formed dentine or walls of the pulp
cavity, after removal of the pulp. For when the pulp is drawn

out of the tooth, the line of separation takes place as a rule between the odontoblasts and the pulp, the former remaining in connection with the dentine.

Figure 5, Plate V, gives a number of forms, not unfrequently observed. A very typical one is *a*, where we observe a large nucleus near the inner rounded end, while the other extremity of the cell looks squarely cut off, with a process or fibres attached to one corner. In other odontoblasts a large process runs from the outer extremity of the odontoblast, evidently pulled out from a dentinal canal, (and as we shall notice afterwards, continuous with the dentinal tubule and its contents), I have never observed two odontoblasts joined end to end.

(6). In a well-stained specimen not only the odontoblasts are of a red color but also the contents of the dentinal canals ; just as in the cornea we have the nucleus, the body of the cell, and its processes by which the protoplasm of the different cells is put in connection, so in the living and growing dentine we have the nucleus in the odontoblast, the body of the cell and its processes permeating the dentine.

(7). If a section is made with a scalpel through the root of the tooth, or more accurately through the dentine with the odontoblasts attached and in place, and such a section is treated with strong hydrochloric acid, the ground substance of the dentine is destroyed, and there are left behind the cells and their main processes, corresponding to the tubules. I may just recall here, that when dead and dry dentine which has been boiled, and where the protoplasm has been destroyed, is treated with strong hydrochloric acid, the tubules remain behind. By taking these two observations together, we see that the odontoblast and the dentinal tubule with its contents are one thing. (It is hardly necessary to mention that the former observation has been made also before by Lent, and the latter by every body.) It is found further that the odontoblasts do not separate readily laterally but are evidently united one with another along their sides, although the connecting fibrils or tubules cannot be distinguished ; but the short processes apparent on the isolated odontoblasts seem to be these connecting threads.

(8). Treating a section prepared as described under (2) with dilute hydrochloric acid and pressing it with a coverglass one often succeeds in separating the odontoblasts—adhering then to

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the pulp—from the dentine, in such a way that fibres are seen across the interval between dentine and soft parts; and in favorable specimens it can be made out that these threads correspond to the large and thick external processes of the odontoblast described above. That they can undergo so much stretching, as they do, without tearing, seems to show that they are not protoplasm pure and simple, but that their outer part is a thin dense envelope, in fact the dentinal tubule (or Neumann's sheath) of the dead and dry dentine. I think some authors confound this elastic tubule with its protoplasmic contents.

Taking all the observations together we would have then—the vascular pulp with its branching cells, the processes of which have no definite arrangement but pass into a fibrous texture, the meshes of which are filled up with a mucous ground substance; and outside the vascular pulp the odontoblasts, the end processes of which pass into the walls of the dentinal canal, *i. e.*, the dentinal tubule. The odontoblasts themselves staining readily and carrying a large nucleus are evidently in great nutritive activity, and their protoplasm is continuous with that lying in the dentinal tubule. The newly formed dentine we find as an apparently homogeneous, semi-transparent coating, covering the calcified dentine; it is not found between the odontoblasts, but only at their outer extremities.

Now taking the case before us, *i. e.*, a root of a tooth which is growing, and waiving at present the question as to the method of the first beginning of the growth of dentine, in what way does the increase in the thickness of the dentine take place?

Taking all the facts into consideration, the most probable view seems to be this: The odontoblasts absorb from the pulp the necessary nutriment and form a secretion; they pour this out in such a way that the portion produced by the single cell cannot be distinguished from that produced by its neighbors, and this new layer stains pink if the lime-salts have not yet been deposited in it. As the odontoblasts form this secretion on their outer ends, they move necessarily inward, and at the same time spin in their wake the dentinal tubule. The side branches of the main tube correspond to the lateral processes (spoken of above) holding the cells together. Of course we must conceive that new lateral processes are continually being formed by the soft anterior part of the odontoblast as this moves and grows inward. In moving onward thus, the odontoblasts must of course remove the pulp, and we may imagine

this to be done in two ways: either the odontoblasts being very active in their nutrition take away the pabulum from the other pulp-cells—the latter shrivelling and disappearing, or the odontoblasts live on the pulp-cells directly just as the tooth-sac of the second tooth absorbs the roots of the deciduous tooth.

Waldeyer has come to different conclusions with reference to this process. In Stricker's Handbook, (p. 337), we find the following passage: " Whilst the peripheric portions of the odontoblasts continually undergo metamorphosis, with disappearance of their nuclei, into a gelatinous matrix which subsequently undergoes calcification, their centric portions penetrate the hardened mass in the form of longer or shorter threads, and represent the first rudiment of the dental fibres. The lateral processes of the odontoblasts occasion the numerous anastomoses of the dental fibres or of the dental tubule. Every odontoblast communicates with the more deeply situated and successively enlarging cells of the young pulp, by means of its pulp process, so that when an odontoblast is calcified up to the base of the fibre another occurs in its place without any interruption to the continuity of the fibre. Hence every dental tubule with its anastomoses must be regarded as formed of several continuous odontoblasts. The layers of matrix immediately surrounding the fibres undergo conversion, as appears from their chemical character, into elastic tissue and form the dental sheaths of Neumann. It has not yet been ascertained whether they also undergo calcification. Thus it appears, that the dentine with all its constituents proceeds from odontoblasts that have become metamorphosed in their form and chemical composition."

There seem to be several objections to this view.

In the first place if we do what Waldeyer asks us and imagine the process to take place as he describes it, let every odontoblast have a pulp-process analogous to its dentinal process—(which I have failed to find and others fail to mention)—imagine the numerous nuclei to disappear, the rearrangement of the cell-processes of the pulp-cells into the tubule-network of the dentine, the metamorphosis of the bodies of the pulp-cells into dentinal matrix, having done that, would we then after all have such a tissue as we find dentine to be? No, we would have a hard tissue, with canals, (but could they have the regularity of the dentinal tubules?) and supplied very richly with bloodvessels something like very vascular bone. Waldeyer quite forgets to dispose of his vessels and they are present in

great abundance. Nor would they disappear by mere conversion of the pulp-cells into gelatinous matrix and Neumann's sheaths.

Further, if such a direct transformation of the pulp took place one might expect to find evidences of the former pulp-structure in the final dentine; this has so far not been demonstrated.

In the third place, if the outgrowths of the dentine took place ought we not to see the deposition of dentine between the odontoblasts along their sides, and ought we not to find dwindling odontoblasts or evidences of disappearance of their nuclei, as well as pulp-cells which were being changed into odontoblasts to take their place; finally, ought there be that tendency to separation between odontoblasts and the remainder of the pulp which certainly exists?

What we really find is the newly produced dentine deposited as a homogeneous coating on the calcified dentine, without any evidence whatever of one portion being the metamorphosed odontoblast and of the other being another changed cell.

If it could however be shown that there was a very intimate union between the odontoblasts and pulp, and if the odontoblasts which Waldeyer figures were the typical ones, this would speak in favor of his view.

It is of course difficult to give convincing proof of such processes as we cannot watch during their occurrence; all we can see is the machine at rest. It seems however to me that there are more difficulties connected with Waldeyer's than with Kölliker's theory.

There is one point however in Kölliker's description with which I cannot agree, namely, the formation of the side tubules. He says: "The finer processes of the dentinal fibril are not present when the dentine is first formed and must be looked upon as secondary formations, just as those of the lacunae of bone."

This point will be better discussed when the formation of the osseous tissue is under consideration.

II. Osseous Tissue.

In the investigations of the formation of osseous tissue, the long bones of kittens, newly born and of more advanced age, were chosen, and the calf's teeth illustrating the formation of cementum, which I include here under bone. Embryonic bones of sheep

and calves were also used, and the tissues were treated essentially as were the teeth for the study of the development of dentine.

Figure 5, Plate V, represents a longitudinal section of a kitten's femur, passing through bone and the outside periosteum. The following are the points that are to be distinguished and taken into consideration. (a) is the fully developed bone substance; in it we recognize the lacunae and canaliculi. The latter (the canaliculi) we cannot see in the *pink zone* (b) although two lacunae happen to be therein, in this specimen. In the soft parts on the outside of the bone we find an outer part, which is distinctly fibrous,—(treatment with strong acids indicate that the fibres are elastic enveloped in a gelatinous homogeneous substance) and an inner part (c) which abounds in (young) cells and which shows but faint fibrillation: (e) is a pink zone similar to (b) and adjoining an Haversian canal, in the lumen of which there appear also a group of cells similar to (c). The soft tissue surrounding the bone, blends with it, merges or passes into it, and we fail to see here such a schematic arrangement of cells (typical osteoblasts as they are described in the books) and which we are led to expect. The specimen was magnified about 500 diameters (Gundl. V. Eyep. III), and reduced in the drawing.

(2). Scraping the surface of such a bone, which has been kept in bichromate of potass solution after the periosteum is removed, we get these covering cells off the bone in a very natural condition, and Figure 6, Plate V, shows some of them. They are all nucleated, which can be demonstrated by the aid of acetic acid. The nucleus was not apparent on all when the drawing was made. Generally short processes are seen, and the drawings show the coarser ones. Finer processes would of course be very apt to be broken off.

(3). Figure 7, Plate V, is a section through part of the root of a calf's tooth showing the cementum and pericementum. We observe here, as in the kitten's femur, the calcified tissue with its lacunae and the processes from these and a very broad zone of uncalcified cementum with numerous lacunae, no canaliculi however are apparent to the eye; just as in the layers b and e from the kitten's femur. As in the periosteum, we find an inner finely fibrillated part of pericementum, rich in cells, and an outer with coarser fibres. The union of the enveloping parts to the tooth is also very intimate. (In parenthesis I may remark, that in speak-

ing of fibrillated or fibrous tissue I am using only descriptive
language; fibres and tubules are not so easily distinguished).

(4). Figure 8, Plate V, is a highly magnified drawing, a por-
tion of a transverse section of a femur of a kitten some months old.
The bones had been remaining in the staining fluid a long time, and
thus one point of importance is brought out plainly.   While in
Figure 5, as well as in Figure 7, we see the pink zone quite
homogeneous, we perceive here that darkly stained lines pass
through it which we may be allowed to interpret as the future
canaliculi.

(5). Treatment of such a section as shown in Figure 7 through
the tooth, with strong muriatic acid brings out other important
facts.   The strong acid will here as elsewhere dissolve the homo-
geneous gelatinous ground substance, it does this in the calcified
part as well as in the pink zone, and in so doing brings to light
in the pink zone a network of fibres and tubules corresponding to
the canalicular network of the calcified cementum.   In fact after
treatment with acid the calcified and uncalcified layers become
one; the walls of the tubules, as in dentine, evidently correspond
to a substance of the nature of elastic fibre.

The same observation can be made on the bone and periosteum.
Acid shows that the pink zone is not homogeneous, although it
appears so to the eye.   In the bone and periosteum another fact
is brought out by this reägent.   After it has acted some time
glistening fibres make their appearance in the periosteum and, by
pressing on the coverglass, one can make out that some of these
periosteal fibres enter the bone.

Taking all these facts into consideration one may form the fol-
lowing conception of the process taking place here on the outside
of the bone, or wherever bone is formed, and on the root of the
tooth.   The cells in the deep layer of the periosteum, or of the
pericementum, multiply and form blood vessels; as they do so they
remain in connection with their mother cells and in all probability
form new connections with neighboring cells; these connecting
processes afterwards become the canaliculi.   In their vital processes
these cells jointly excrete a gelatinous material and the elastic
membranes, which partly if not altogether produce the striation
observed in bone and so plainly visible in cementum; the newest
layer presents itself, when treated in the way indicated, as
the pink zone; as the cells secrete layer upon layer they, as a

whole, are carried outward further away from the finished bone-substance. Some of the cells however, get entangled—so to speak—in the secretion, and come to be, in the fully formed bone, the lacunae, (or at least their contents). At the same time as the layer of plastic cells moves outward, secreting the basis-substance, they spin out, or draw out their processes thus giving rise to the canaliculi. Although these under ordinary circumstances are not easily recognized, they are already present from the beginning and are formed *pari passu* with the ground substance of the bone. All it needs to make them apparent is the infiltration of the newly formed tissue with lime salts.

One may compare the surface of a growing bone with that of a granulating ulcer; on the surface proliferation of new cells and formation of new blood vessels takes place (only in the bone they are a wide network while in an ulcer they form loops), and a little deeper in the deposition of new substance takes place; in the one case the typical osseous tissue and in the other the cicatricial substance.

The view presented here on osteogenesis allows also enough liberty for the formation of the different varieties of bone, which vary, *e. g.*, not unmarkedly in the young and the old, the character of the bone depending on the nature of the fibrillar or connective tissue forming it.

Kölliker and Virchow offer a different explanation of the formation of the canaliculi. Kölliker says, p. 222, 5th Ed., 1867, of his histology: "According to Virchow's discovery, which I can fully confirm, these cells [the periosteal cells] become stellate gradually, and are thus changed directly into the stellate bone corpuscles."

Virchow gives the following explicit account, p. 469, Cell. Path., 7th Am. Ed.: "The cartilage cells (and the same holds good of the marrow cells) during ossification throw out processes (become jagged) in the same way that connective tissue corpuscles, which we also originally found, do both physiologically and pathologically. These processes which in the case of the cartilage cells are generally formed after, but in that of the marrow cells frequently before, calcification has taken place, bore their way into the intercellular substance like the villi of the chorion do into the mucous membrane and into the vessels of the uterus, or like the Pachionian granulations (glands) through the calvarium."

"The cells which thus result from the proliferation of the peri-osteal corpuscles are converted into bone corpuscles exactly in the way I described when speaking of the marrow. In the neigh-borhood of the surface of the bone the intercellular substance grows and becomes almost cartilaginous. The cells throw out processes, become stellate and at last the calcification of the intercellular substance ensues."

A view on the formation of osseous tissue differing from the one above worked out, is that of Waldeyer, which is gaining favor among histologists.

"The osteoblasts," says Waldeyer, "are the embryonal cells forming the osseous tissue, a portion of the same (the nucleus dis-appearing) is changed into a gelatinous more or less fibrous texture, which during normal ossification takes up lime salts almost at the same time; of a certain proportion of these osteoblasts only the peripheral part of the protoplasm is thus changed, what is left remains behind as the nucleated bone corpuscle, imbedded in the intercellular substance, like a connective tissue corpuscle in the substance of tendon."

After describing the calcified cartilage and the changes it under-goes, Waldeyer gives a description of the parts in which the first deposition of bone takes place (the crypts of calcified cartilage with the medulla), which seems to me true and to fit into my theory fully as well or better than into his.

On p. 365 of the Archiv für Mikroscopische Anatomie, I, 1865, he makes a statement which I cannot bring in harmony with his Figure 2. He says: "At the time when the first bone substance is deposited upon the cartilaginous framework, there is not the trace of a separation to be observed between the osteoblasts and the medullary tissue. This occurs later, when a very distinct stratum of bone is deposited."

But on looking at the drawing we see a marked difference between the fibrous tissue in the centre of the cavity and the layer of "osteoblasts" lining the walls of calcified cartilage, no bone having as yet been laid down there.

Waldeyer continues his argument thus: "It is not difficult to ascertain here already the correctness of my view regarding the formation of osseous tissue, as I expressed it above. While the first bone substance is formed the medullary spaces are closely filled with osteoblasts, there is no room left for any excretion,

excepting that at the same time a number of osteoblasts perish, which cannot be assumed, or a bone substance ought to be formed studded so thickly with lacunae as it is never found to be the case. This fact makes it to me very improbable that the ground substance of bone is a mass excreted by the osteoblasts."

The examination of these regions has never roused this difficulty in my mind, and if we again turn to the figures of Waldeyer himself, we find no lack of space, there is amply room in the spaces produced by the openings of the cartilage cells for six times as many cells as are figured—hence also for a thin coating of bone.

Waldeyer continues: " One observes further that the peripheral parts of the single osteoblasts are changed, loosing their darkly granular appearance and applying themselves closely to the sinuous walls of the medullary spaces. Other osteoblasts in the neighborhood are in connection with these modified peripheral layers, they also with their metamorphosed outer layers approaching the former. The portion of protoplasm around the nucleus only remains unchanged. I take this change of the peripheral strata for the expression of a metamorphosis into glue yielding substance, which at once takes up the lime salts."

Looking at the figures, I fail to see any indications of such processes, especially can I not distinguish between unchanged osteoblasts and such as are dwindling, their bodies being changed into gelatinous substance. Reading this description one would also think that the deposition of new osseous substance was taking place all around the cells, around each individual cell, and that the substance was immediately calcified ; in fact one would expect a tissue somewhat like cartilage. But neither is the case, neither the laying down of bone substance around individual cells nor the immediate calcification. The new layer is in sections and always forms an uncalcified seam, in which here and there a "cell" is found.

Although there are disadvantages connected with the carmine method, as above described, yet there are some facts brought out by it very well ; and the individual steps of the processes can I think be better followed than by the chromic acid method. Using chromic acid and decalcified tissue there are certain differences necessarily obliterated, which the other method brings out, and which are apt to make a strong impression.

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That the cells alter in character would be not easy to prove, as it depends on very slight changes in size, and the greater or less amount of granules, but if it should be the case, this fact would favor the theory supported in this paper very well indeed, as I shall show further on.

So does also the fact, that the cells in specimens which have been brushed, are partly free, partly adherent to the bone by their processes.

Waldeyer further points out, that the cells inclosed in the osseous substance are smaller than the osteoblasts, and says: "If we find however cells in formative action, it is difficult to conceive how they can effectually perform their functions, and at the same time undergo atrophy."

This difficulty can easily be overcome by the examination of gland cells which have been resting, and such as have been made to secrete very actively; the former we find large, plump, with sharp outlines; the latter small, shrunken, shrivelled, their outlines difficult to make out. We see here that cells which are secreting actively shrink very markedly; and might such a change not have been expected?

I said above that it was not easy to distinguish with certainty minute differences in size. To support this statement, I would call attention to a figure, by Klein, in Sanderson's Handbook, a transverse section of a femur from a human fœtus, treated with chromic acid; I do not think that any one can perceive any difference in size between the cells lining the medullary spaces and those inclosed in the lacunae.

To explain the process as taking place beneath the periosteum, Waldeyer adduces a cross-section of a fœtal Tibia, (Plate XXII,* Figure 5)—at (a) we are to see osteoblasts. I have serious doubts, however, if at this place, and others, where Waldeyer thinks deposition of new osseous tissue is taking place, *the opposite is not occurring, namely, the absorption of the bone.* There is a good deal of evidence that the latter is the case,—the jagged outline and characteristic excavations,—while there is no evidence at all that formation is just now taking place there. First there ought reasons to be given that formation is going on there at all before the specimen is used for demonstration. Treatment of the material with carmine

* Arch. f. Mik. Anat., 1865.

in the way described shows at once where excavation and absorption is going on, where deposition of new bone is going on, and where the soft parts covering the osseous tissue are at rest.

The view here favored agrees with Kölliker's, excepting as regards the formation of the canaliculi, and probably agrees with Gegenbaur's, (whose writings I have not had opportunity to examine) if I may form a judgment from scattered references.

DESCRIPTION OF PLATE.

FIGURE 1.—Section through root of calf's incisor. *a*, pulp; *b*, uncalcified dentine; *c*, dentine; *d*, cementum; *e*, uncalcified cementum; *f*, pericementum.

FIGURE 2.—Section through pulp, odontoblasts and dentine; calf's incisor. *a*, dentine; *b*, uncalcified dentine; *c*, odontoblasts; *d*, pulp.

FIGURE 3.—Odontoblasts from calf's tooth.

FIGURE 4.—Pulp-cells from same.

FIGURE 5.—Section through femur and periosteum; kitten at birth. *a*, fully formed bone; *b* and *e*, uncalcified bone; *c*, layer of cells forming bone; *d*, outer periosteum.

FIGURE 6.—Osteoblasts; cat's bone.

FIGURE 7.—Section through cementum and pericementum of calf's tooth. *a*, cementum; *b*, uncalcified cementum; *c*, cementum forming cells; *d*, outer part of cementum.

FIGURE 8.—Section through femur, kitten 3–4 months, deeply stained.

All drawings except Figure 1 were made under Gundlach V. Oc. 3, and reduced.

THE FIRST ZOEA OF PORCELLANA. By W. K. BROOKS and E. B. WILSON. With Plates VI and VII.

Since 1835, when Thompson obtained the larva of a British species of Porcellana from the egg, this very remarkable zoea has frequently attracted the attention of naturalists, and we now have quite an extensive list of papers, giving a satisfactory account of the structure of the advanced zoea, and of its transformation into the adult crab. The bibliography of the subject is given, at length, in a recent paper by Faxon, (On some young stages in the development of Hippa, Porcellana and Pinnixa. Bulletin of the Museum of Comparative Zoölogy, at Harvard College, Vol. V, No. 11,) and it seems unnecessary to duplicate it here.

Most of the observers who have studied it started with the advanced zoea which is frequently captured with the hand net at the surface of the ocean, and the few papers which notice the early stages of the larva were published so long ago, that a minute account of the young, as it leaves the egg, is still lacking.

During the latter part of June, 1880, we obtained, at the marine laboratory of the Johns Hopkins University, at Beaufort, N. C., a female specimen of *Porcellana ocellata*, Gibbes, with eggs, which we succeeded in keeping alive and in good condition until the eggs hatched, and we were thus supplied with an abundance of material for studying the early stages.

As all the members of the party were at the time fully occupied with other work, we undertook to study the larva together, and to make as many notes and drawings of the early stages as possible.

This paper is therefore the result of our combined observations, but the work of copying the original drawings, and of preparing the description has been done by W. K. Brooks. In the explanation of the figures the author of the drawing which was copied is named in each case, although in nearly every case, the accuracy of the observation was verified by an independent drawing by the other observer.

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The larva immediately after its escape from the egg, is shown in Plate VI, Figure 1. It is able to rise from the bottom and to swim a little by flapping its abdomen, but until the next moult it spends most of its time lying nearly motionless upon the bottom.

The carapace makes a little more than two-fifths of the total length of the body, and is folded upon itself in such a way as to form a well defined transverse band running across its dorsal surface near the posterior edge. The posterior spines of the carapace do not seem to be at all invaginated, but they are very much convoluted and wrinkled, and their free extremities are bent forwards under the posterior edge of the carapace. Between the eyes the anterior end of the carapace forms a protuberant rounded front, and the convoluted and wrinkled rostrum is bent down towards the ventral surface. The eyes lie in deep notches on the anterior edge of the carapace, and they appear to be movable, although the stalks are very short.

The third pair of maxillipeds are small and rudimentary, while the first, *Mp*, and second, *Mp'*, pairs are well developed, although their locomotor setæ are not yet protruded, and the limbs are not moved but remain constantly in the position which is shown in the figure. The abdomen has five free movable somites, besides the sixth which is not separated from the telson, *T*.

The pigment is more conspicuous at this time than during the stages which follow, and consists of a number of pretty constant bright red spots. One of them is on the basal portion, and one on the flagellum of the second antenna, one on the mandible, *M*, one on the basal joint of the first maxilliped, two on the basal joint of the second and one on the third, as well as one about half way between the base and tip of the second; there is a long dendritic spot on the posterior edge of the first, the second, the third, and the fourth abdominal somite, and a pair of spots on the telson.

The whole surface of the body is covered by a delicate embryonic cuticle, which is too transparent to be visible with the magnifying power under which Figure 1 was drawn. This cuticle conforms to the outline of the body except on the two pairs of antennæ and the telson. It will be described, in detail, later, in the account of the appendages.

Some of the larvæ free themselves from it within a couple of hours, and assume the form shown in Plate VI, Figure 5, while others do not escape from it until nearly or quite twenty-four hours

after they leave the egg. After this first moult the stalks of the eyes, (see Figure 5), elongate, the fold at the posterior edge of the carapace is stretched out so that the latter is now about half as long as the whole body; the rounded front disappears, and the convolutions and wrinkles of the rostrum and spine are no longer seen, although these processes are still rolled up, as shown in the figure. Figure 5 shows them as they appeared in the zoea which was drawn, but the form of the bends is not at all constant.

The swimming hairs on the first and second maxillipeds, Mp, Mp', are extended, and these appendages, as well as the telson, are now used as locomotor organs. Spines have now made their appearance upon the posterior edges of the third, fourth and fifth abdominal somites, and the rostrum and processes of the carapace are covered with short hairs.

In from one to two days after hatching the rostrum and processes become extended, as in Plate VII, Figure 8, and the zoea assumes the familiar form which has been described and figured by many observers.

The Appendages:

The first antenna of the newly hatched larva is shown in Plate VI, Figure 2, and that of the fully developed zoea in Plate VII, Figure 3.

In the first stage it is covered by the delicate embryonic skin, which follows the outline of the appendage very closely, except at the tip where it is produced into two long, broad, flattened, pointed setæ," which are fringed with smaller hairs. These structures, which seems to be swimming hairs, are not present in the zoea after the moult, but in the first stage the antenna carries a single stout sensory hair which, as shown in Plate VI, Figure 2, extends into one of the swimming hairs, more than half way to the tip. After the moult, Plate VII, Figure 3, the appendage ends in a number of long blunt sensory hairs, from the bases of which fine fibres run downwards to a large club-shaped granular mass, which appears to be ganglionic in nature.

The second antenna is shown before the moult, in Plate VII, Figure 1, and after the moult in Plate VII, Figure 2. It is essentially alike in both stages, but before the moult is loosely invested by the embryonic skin, which is loose and much larger than the true appendage. It consists of a swollen basal portion d, which carries a short pointed external branch, and a longer internal branch.

The mandibles and maxillæ are shown before the moult in Plate VII, Figure 7, and after the moult, in Plate VI, Figures 3, 4, and Plate VII, Figure 5.

In the first stage, Figure 7, Plate VII, these three appendages are folded together, and covered by the embryonic skin which is nearly conformable to their surface, although, as shown by the light outer line in the figure, it does not follow all the folds. No trace of a palpus could be discovered on the mandible, and the hairs at the tip of the maxillæ were almost completely invaginated into the appendages.

After the moult these three pairs of appendages become functional, and have nearly the adult character. The mandibles, Plate VII, Figure 5, and Plate VI, Figure 6, M, are not exactly alike, but exhibit that slight departure from bilateral symmetry so frequently found in these appendages. No trace of a mandibular palpus could be found, although there was a small area where the integument had been broken in each of the two specimens which were dissected; and as this area, shown in the figure, was at the same place in both cases, the fracture may have been produced by the removal of a palpus.

The first maxilla, Plate VI, Figure 3, and Figure 6, Mx, consists of a two-jointed basal portion, a, b, with stout cutting hairs, and a slender endopodite c, which in one specimen ended in two, and in another specimen in three long, slender, irregularly plumose hairs. The distal joint, b, of the basal portion carries upon its cutting edge, one row of five stout spines and a second row of four slender spines parallel to the larger ones. In the specimen figured, the proximal joint, a, was twisted so that its inner surface was shown, and the posterior edge is therefore the one at the left of the figure. It carries five long, stout, plumose spines, and at the posterior angle of its cutting edge a single spine without secondary hairs. No trace of an exopodite or scaphognathite could be detected in this appendage.

The second maxilla, Plate VI, Figure 4 and Figure 6, Mx', consists of a three-jointed basal portion with short stout hairs; a two-jointed endopodite, b, with longer hairs; and a long flat exopodite, c, with five long hairs at its distal, and a long plumose flagellum, d, at its proximal end.

In the first stage, the first and second maxillipeds, Plate VI, Figure 1, Mp, Mp', are fully developed, although the presence of

the embryonic skin prevents the extension of the locomotor hairs.

In Figure 1, the rudimentary third maxilliped is shown behind the base of the second.

In Plate VII, Figure 4, the third maxilliped, c, is shown, more highly magnified, lying in the same series with the bases, a and b, of the first and second. A fourth appendage, no doubt the first pereiopod, is also represented at this stage by a bud or rudiment, d, and the appendages, b, c, and d, are furnished with little buds, which would seem to be rudimentary gills. After the moult we were not able to detect either the appendage, d, or the gill-like processes.

After the embryonic skin is moulted, the locomotor hairs of the first and second maxillipeds lengthen and these appendages become functional, while the third pair remain rudimentary. Figure 6, Plate VII, shows the first and second maxillipeds soon after the moult, and hardly calls for explanation.

The embryonic skin conforms closely to the surface of the abdomen and telson, although it appears to have no trace of a division into somites.

Figure 7 of Plate VI shows one-half of the telson of Figure 1 before the embryonic skin is shed. A comparison with Figure 6, T, will show that the great difference which has been pointed out by Faxon and others between the telson of the embryonic skin and that of the zoea in the ordinary crab, does not occur in Porcellana, but that the two are here nearly alike.

The five pairs of long swimming hairs of the zoea are, before the moult, about half invaginated, and the extended portion, Plate VI, Figure 8, is finely plumose. The hairs of the embryonic cuticle are much stouter, and their edges are not plumose, but they agree with those of the zoea, in number and arrangement.

The outer hair, or marginal spine of the telson, has the same appearance before the moult that it has afterwards.

EXPLANATION OF THE FIGURES.

PLATE VI.

FIGURE 1.—Zoea immediately after its escape from the egg, seen from the left side. From a drawing by W. K. Brooks.

FIGURE 1.—*Continued.*

> *A*, first antenna; *An*, second antenna; *M*, mandible; *Mp*, first maxilliped; *Mp'*, second maxilliped; *R*, rostrum; *T*, telson.

FIGURE 2.—First antenna of the same larva, more highly magnified. From a drawing by W. K. Brooks.

FIGURE 3.—First maxilla of the larva shown in Figure 5. From a drawing by W. K. Brooks.

> *a*, proximal joint of basal portion; *b*, distal joint of basal portion; *c*, endopodite.

FIGURE 4.—Second maxilla of the larva shown in Figure 5. From a drawing by W. K. Brooks.

> *a*, three-jointed basal portion; *b*, two-jointed endopodite; *c*, scaphognathite; *d*, flagellum.

FIGURE 5.—Zoea, seen from the right side, immediately after moulting the embryonic skin. From a drawing by E. B. Wilson.

> *A*, first antenna; *An*, second antenna; *Mp*, first maxilliped; *Mp'*, second maxilliped; *R*, rostrum.

FIGURE 6.—Ventral view of the same zoea, one day after moulting the embryonic skin. From a drawing by E. B. Wilson.

> *A*, first antenna; *An*, second antenna; *L*, labrum, *M*, mandible; *Mp*, first maxilliped; *Mp'*, second maxilliped; *Mx*, first maxilla; *Mx'*, second maxilla; *R*, rostrum; *T*, telson.

FIGURE 7.—Dorsal view of right half of telson of the larva shown in Figure 1. From a drawing by E. B. Wilson.

FIGURE 8.—One of the setæ of Figure 7, more highly magnified. From a drawing by E. B. Wilson.

PLATE VII.

FIGURE 1.—Second antenna of the larva shown in Plate VI, Figure 1. From a drawing by W. K. Brooks.

> *a*, embryonic skin; *b*, external branch; *c*, internal branch; *d*, enlarged basal joint.

FIGURE 2.—Second antenna of the zoea shown in Plate VI, Figure 5. From a drawing by W. K. Brooks. Letters of reference as in Figure 1.

FIGURE 3.—First antenna of the zoea shown in Plate VI, Figure 5. From a drawing by W. K. Brooks.

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FIGURE 4.—Basal joints of the maxillipeds of the larva shown in Plate
VI, Figure 1.   From a drawing by W. Brooks.
a, base of first maxilliped; b, base of second maxilli-
ped; c, third maxilliped; d, first pereiopod; e, edge
of carapace.

FIGURE 5.—Mandible of the zoea shown in Plate VI, Figure 5.   From
a drawing by E. B. Wilson.

FIGURE 6.—First and second maxillipeds of the zoea shown in Plate
VI, Figure 5.   From a drawing by W. K. Brooks.

FIGURE 7.—Mandible and maxilla of the larva shown in Plate VI,
Figure 1.   From a drawing by W. K. Brooks.
M, mandible; Mx, first maxilla; Mx', second maxilla.

FIGURE 8.—Side view of the zoea, one day after moulting the embryo-
nic skin.   From a drawing by E. B. Wilson.

ERRATA.

Page 60, bottom line,
for "external," *read* "internal."
for "internal," *read* "external."

THE STUDY OF HUMAN ANATOMY, HISTORICALLY AND LEGALLY CONSIDERED.[1] By EDWARD MUSSEY HARTWELL, M. A., *Fellow of the Johns Hopkins University.*

PART FIRST.

"Practised architects, before they venture in thought to build a new edifice, to strengthen an old one, or restore a ruined one, first consider carefully and examine closely all the minute parts of such structures. So, physicians, indeed, before they endeavor to care for the human body and preserve it from the diseases which threaten it, ought to know very accurately, and to a nicety, all the parts of that body. Anatomy, the eye of medicine, furnishes such knowledge. Verily, the beginnings, the foundations, and the sources of origin of the medical art are, without the light and vision of anatomy, shrouded in thick darkness; wherefore, it is not inaptly called by Johannes Montanus, the alphabet of medicine." So wrote Rolfincius, in his "Dissertationes Anatomicæ," published at Nuremberg, in 1656.

When we of to-day seek the origin of this "alphabet of medicine," we turn to the East, whence we are accustomed to derive the beginnings of all our arts; but we find the history of ancient anatomy to be almost a blank page. Priest, and law-giver, and people were all averse to anything like the dissection of the human body. The Egyptians, Hebrews, Greeks, Romans, and Arabs, alike regarded with abhorrence the mutilation of the dead. There is abundant proof of this in their laws and customs touching burial and defilement.

It is said that Democritus, of Abdera (460 B. C.), the friend of Hippocrates, was the first to dissect the human body. However

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[1] Portions of the following paper have been printed already in the Journal of the American Social Science Association; the Boston Medical and Surgical Journal and the Brooklyn Annals of Anatomy and Surgery. In its present form it contains much new material; and embodies the result of the latest statistics and most recent legislation so far as I could ascertain.

Galenism died hard, even in Italy where it was first attacked. How tenacious it was of life is well shown by Malpighi, who was born in 1628, the year that Harvey first published his "Essay on the Motion of the Heart and Blood." Harvey never saw the passage of the blood through the capillaries; Malpighi discovered those vessels and first demonstrated the flow of blood from the arteries into the veins. Malpighi writes: "In the meantime, contentions being raised among studious men, especially the younger, both practical and theoretical, and the new doctrines growing daily into more credit, the senior professors of Bologna were inflamed to such a pitch, that, in order to root out heretical innovations in philosophy and physic, they endeavored to pass a law whereby every graduate should be obliged to take the following additional clause to his solemn oath on taking his degree, viz: 'You shall likewise swear that you will preserve and defend the doctrine taught in the University of Bologna, namely, that of Hippocrates, Aristotle, and Galen, which has now been approved of for so many ages; and that you will not permit their principles and conclusions to be overturned by any person, as far as in you lies.'" "But," says Malpighi, "this was dropped and the liberty of philosophizing remains to this day."

Practical anatomy was taught at Padua it is said, as early as 1151; Haeser, in the third edition of his Geschicte der Medecin says that: "in the year 1238, Kaiser Frederick II. ordered, at the suggestion of Marcianus, chief physician of Sicily, that every five years a corpse should be dissected publicly, and that physicians and surgeons should be admitted, according to their rank, to the dissection." It is elsewhere stated that Frederick forbade, in the code for Sicily, any one to practice surgery unless he had been instructed in anatomy. There is no dispute, however, that Mondino publicly dissected two subjects as early as 1315; and some writers give 1308 as the date.

We find many bulls of Popes and canons of Councils regarding the study and practice of physic and surgery by monks; from the time of the Council of Laodicea, in 366 A. D., when the priesthood were forbidden to study enchantment, mathematics, and astrology, and the binding of the soul by amulets, till 1215, when Pope Innocent III. is said to have fulminated an anathema against bloody operations in surgery. Although these utterances of the Church are interesting, we pass them by as being outside the scope of this paper.

The edict of Boniface VIII., however, published in 1300, affected the progress of practical anatomy, and is worthy of note. In 1299, Pope Boniface VIII. forbade, under pain of excommunication, any one to boil, cut up, or dry the bodies of the dead. Such an act he characterized as barbarous and abhorrent to Christian piety. Raynaldus, in whose " Annales Ecclesiastici, Lucae, 1749," the edict of Boniface is found, says that such customs " had prevailed in regard to those who, having undertaken a pilgrimage to the East, died in foreign parts; and in order that their bones might be freed from flesh, and so easily carried about without the fear of corruption. And yet we know," he adds, " that the body of Saint Luke was boiled by his friends." It is hardly probable that Pope Boniface directed this edict primarily against anatomy. Edward I., of England, directed that the flesh should be boiled from his bones and that they should be carried to battle in a bag by his successor, in order to terrify his enemies. The story of Douglas and the heart of Robert Bruce is familiar to all. It is quite likely that Boniface launched his anathema in order to restrain such practices as these; nevertheless, his edict proved an obstacle to anatomical studies. Mondino apologizes for not making the most exact study of the bones of the skull, saying : " the bones beneath the basilar bone are not to be clearly distinguished, unless they be boiled ; a sin which I have been accustomed to shun." Hyrtl, the famous German anatomist, holds that the edict of Boniface was in force till 1556, when the Emperor Charles the Fifth, the patron of Vesalius, ordered the question to be put to the theologians of the University of Salamanca, " Whether or not it could be allowed, without violating one's conscience and incurring the suspicion of criminality, to cut up human dead bodies ? " " *Et respondisse Universitatem, Licere.*" says Rolfincius, quoting a still earlier writer.

That dissection was not universally banned by the Church before the Divines of Salamanca pronounced it lawful, may be seen from the action of Pope Sixtus IV., in 1482. In that year, in a letter addressed to the rector, doctors, and students of the University of Tübingen, Sixtus granted a special and full dispensation to those who should receive the cadavera of certain malefactors executed for capital crimes in accordance with the civil law : " *per justitiam secularem,*" is the phrase in the original. They were given permission to dissect and dismember these dead bodies, inasmuch as

they desired thereby to render themselves learned and skilful in
the art of medicine, provided they would bury in the customary
manner such condemned men after they should be dissected and
dismembered.

The Grand Council of Venice, in 1308, passed a decree ordering
the medical college of that city to undertake a dissection once a
year. It is claimed that in Prague, as early as the foundation of
the University in 1348, the executioners were enjoined to deliver
the cadavera of malefactors to the school of medicine. Duke
Albrecht IV. imported an Italian anatomist, named Galeazzo, to
introduce the art of dissection into Vienna; where the first anatom-
ical demonstrations before the medical faculty were made in 1404.

In France, as early as 1376, Louis of Anjou permitted the sur-
geons of Montpellier to take the body of an executed criminal
annually for dissection. Charles the Bad, King of Navarre and
Lord of Montpellier, ratified this grant in 1377; as did King
Charles VI. in 1396; and King Charles VIII. in 1484, and again
in 1496. A similar grant was made in Scotland in 1505, as we
learn from the following extract taken from the Charter given by
the Town Council of Edinburgh to the Surgeons' Company, July
1, 1505, and ratified by King James IV. in the following year:
"And als that everie man that is to be maid frieman and maister
amangis ws be examit and previt in thir poyntis following THATT
IS TO SAY That he knaw anatomea nature and complexioun of
every member In manis bodie. And in lyke wayes he knaw all
the vaynis of the samyn thatt he may mak flewbothomea in dew
tyme. And als thatt he knaw in quhilk member the signe hes
domination for the tyme for every man aucht to knaw the nature
and substance of every thing thatt he wirkis or ellis he is negligent.
And that we may have anis in the yeir ane condampnit man after
he be deid to mak anatomea of quhairthrow we may haf experience
Ilk ane to instruct others And we sall do suffrage for the soule."
By Act of Parliament, 32 Henry VIII., cap. 42, in 1540, it was
granted to the Barber-Surgeons of London to take " yearly forever
. . . . . four persons condemned, adjudged and put to Death for
Felony by the due Order of the King's Highness, ... and to
make Incision of the same dead Bodies, or otherwise to order the
same after their said Discretions at their pleasures, for their further
and better Knowledge, Instruction, Insight, Learning, and Expe-
rience in the said Science or Faculty of Surgery."

Vesalius, a Fleming, born in 1514, did more than all his predecessors to overthrow Galenism and place medicine upon a rational basis, and well deserves his title of the Father of Modern Anatomy. Yet, despite the concessions we have noticed made by prelates, kings and parliaments to the early anatomists, Vesalius and his students were obliged, in the words of Hallam, " to prowl by night in charnel-houses, to dig up the dead from the grave ; they climbed the gibbet in fear and silence to steal the mouldering carcass of the murderer at the risk of ignominious punishment and the secret stings of superstitious remorse." Vesalius began to dissect while a youth in his teens. For a time he studied under the famous French anatomist, Jacques Du Bois, who demonstrated the anatomy of Galen on the carcasses of dogs. But Vesalius forsook Paris for Italy, drawn thither by the reputation of the schools whence Leonardo da Vinci and Michael Angelo derived their knowledge of human anatomy. Before he was twenty-eight, as has been well said, " Vesalius discovered a new world," and held at one time the professorship of anatomy in the universities of Pisa, Padua and Bologna. He died the victim of the Spanish Inquisition. His inspection, with the consent of the relatives, of the body of a Spanish grandee, whose heart feebly contracted under the knife, brought him before the Inquisition, and would have led him to the stake but for the intercession of the King. Compelled to journey to Jerusalem by way of penance, Vesalius was shipwrecked, in 1564, on the island of Zante. It is said that he there starved to death, and that unless a liberal goldsmith had defrayed the funeral charges, the remains of the greatest anatomist the world had seen would have been devoured by birds of prey.

The Italian schools under Vesalius and his successors, Fallopius, Columbus and Fabricius, exerted a wide and potent influence upon European medicine. This influence was sooner felt and more marked in France, Germany and Holland than in England and Scotland. The following statements, made by Billroth, may serve to indicate the favor in which anatomy was held in Germany :

In the Privilegia granted by the Landgrave Wilhelm von Hessen to the University of Marburg, in 1653, it is provided that " in the medical faculty at the start there shall be two doctors in pay, who, in addition to the theory, shall conduct the practice of anatomy and of botany with the youth." The statutes of the

medical faculty at Marburg for the same year, Title IV, read as follows:—

"(1.) It is clear that anatomy, next after psychology, forms the chief part of universal physiology. Since there is a twofold method of teaching it, one that is ordinarily practiced in anatomical theatres in the presence of many spectators, and the other which is employed by the holders of scholastic chairs, let neither of them be intermitted. Let both of them, as well publicly as privately, be practiced.

"(2.) Let also the art of dissection and of skillfully handling and applying the knife in individual parts be shown, in order that a difference may be noted between physical and medical or practical anatomy. The various skeletons, also, both male and female, of common and exotic animals shall be prepared, in order that not only the structure of the skeleton, but also the whole of osteology, may become known to students of medicine as well as of surgery.

"(3.) Let pregnant women be dissected as well as others. Let mid-wives as well as others be admitted.

"(4.) Let not those who are condemned to death be opened alive, but let living things of every kind, as insects, serpents. aquatic animals, birds, and quadrupeds, be dissected. Especially let those studying anatomy observe, more precisely than butchers would, domestic quadrupeds while they are being slaughtered.

"(5.) Moreover, let the bodies of atrocious criminals, whether they have been beheaded or hanged, be designated for dissection. Let them not be kept back by the magistracy when they are sought for this purpose, in order that those who have done as much evil as they could when alive, may, after death, on the other hand, be of as much service and use as possible."

We shall confine our attention chiefly to the history of anatomy in Great Britain; inasmuch as in the development of anatomy in America, the influence of Edinburgh and London is more readily traced than that of Paris and Leyden.

Twenty-five years after the passage of the Act of 32 Henry VIII., Queen Elizabeth granted to the College of Physicians, of London, the bodies of four felons executed in Middlesex, "that the president or other persons appointed by the college might, observing all decent respect for human flesh, dissect the same." In 1663, Charles II. increased the number of felons' bodies, annually

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granted to the physicians, to six. The Act of 22 George II., c.
37, 1752, required the dissection or hanging in chains of the
bodies of all executed murderers in order that "some further Terror
and peculiar Mark of Infamy might be added to the Punishment
of Death." The provision of this Act regarding the dissection of
murderers remained unrepealed till the passage of the so-called
Warburton Anatomy Act, in 1832, while the provision regarding
the hanging of a murderer's body in chains remained in force till
1861, when it was repealed.

These were the only legalized sources for the supply of anatomi-
cal material in England prior to 1832. Such provisions might, at
first sight, seem generous and ample, yet they were not. We find
Dr. William Hunter, in 1763, in vain asking of the King a grant
of land sufficient for the site of an anatomical school in London,
which he proposed to endow with something like £7,000, and one
of the finest anatomical collections in Europe. In his memorial
to the Earl of Bute, Hunter writes: "Of the very few who profess
or teach this art in any part of Great Britain, London excepted,
there are none who can be supplied with dead bodies for the
private use of students. They can with difficulty procure only so
many as are absolutely necessary for the public demonstrations of
the principal and well-known parts of the body. Hence it is that
the students never learn the practical part, and the teachers them-
selves can hardly make improvements, because they cannot have
subjects for private experiments and enquiries. Anatomy was not
upon a much better footing, even in London, till the year 1746."

In 1832, Parliament passed the Warburton Anatomy Act,
which is still in force throughout Great Britain and Ireland—in
all its essential features. To understand its significance and that
of "Burking," which really caused Parliament to enact it; we
must glance at the Edinburgh School of Anatomy.

We have already noticed the grant of anatomical material con-
tained in the charter of the Surgeons' Company, made in 1505.
The beginning of the Edinburgh Anatomical School was in 1694;
when the Town Council, on the 24th of October, in response to
the petition of Alexander Monteith, granted him "any vacant,
waste room in the correction house, or any other thereabouts
belonging to the Town." Monteith also obtained a grant of
"those that dye in the correction house; and the bodies of fund-
lings that dye upon the breast." The Surgeons' Company were

granted, nine days later, "the bodies of fundlings who dye betwix
the tyme that they are weaned and their being put to schools or
trades; also the dead bodies of such as are stiflet in the birth,
which are exposed and have none to owne them; as also the dead
bodies of such as are *felo de se* and have none to owne them; like-
wayes the bodies of such as are put to death by sentence of the
magistrat, and have none to owne them." Certain interesting
conditions were attached to the grants to Monteith and the Sur-
geons. The dissection was to be during the winter, from one
equinox to the other; all the "gross intestines" were to be buried
within forty-eight hours, and the rest of the body within ten days
at the grantees' expense. The regular apprentices of the Surgeons
were to be admitted at half price, and any magistrate who thought
fit might attend the dissection. In the grant to the Surgeons, no
mention is made of the gross intestines, according to Dr. J.
Struthers, from whose sketch of the Edinburgh Anatomical
School these facts are taken; but it is provided "that the petitioners
shall, before the terme of Michaelmas 1697 years, build, repair, and
have in readiness, ane anatomicall theatre where they shall once
a year have ane public anatomicall dissection, as much as can be
showen upon ane body, and if the failzie thir presents to be void
and null." The Anatomical Theatre of the Surgeons was reported
finished to the Town Council, December 17, 1697. The Council
ratified its grant of 1694, and, the same day, the Surgeons chose
a committee "to appoint the method of public dissections, and the
operators." In 1705, the Council gave £15 salary to Robert
Elliott, the first Professor of Anatomy in Edinburgh. In 1720,
the Town Council elected Alexander Monro, *primus*, Professor of
Anatomy. In 1725, he removed from Surgeons' Hall to the
University buildings, because of the violence of a mob which had
attempted to demolish the Surgeons' Theatre, on account of the
supposed violation of graves. In 1722, the apprentices of the
Surgeons' Company were obliged, in their indentures, to subscribe
to "an obligation that they would altogether avoid raising the
dead."

Under the Monros, father, son and grandson, who held between
them the University Chair of Anatomy from 1720 till 1846, the
school became widely famous. Many of the early American phy-
sicians and anatomists studied at Edinburgh; where, early in this
century, there were several extramural private schools of anatomy.

Of these, that of Dr. Robert Knox was the most famous and frequented. In the winter of 1828–29, he had a class of 505: the largest in Europe.

For years the demand for anatomical material had exceeded the legal supply in Great Britain. As early as 1826, Parliament was petitioned, but in vain, to give aid and protection to the anatomists,—who were forced to depend on the resurrection men for subjects. Bodies often brought £10 each, in Edinburgh and London; in one instance a subject was sold for £30. When the home supply ran short, the Scotch anatomists were furnished with stolen bodies from England, Ireland, and even France. "The increased demand and higher pay for material," says Lonsdale, (Knox's biographer), "generated sad recklessness and brutality. Quarrels arose over the spoils; the jealousy of rival factions of the different schools, and the frequent attempts to outwit each other, led to personal denunciations and a fearful publicity." In response to numerous petitions from the medical profession, a "Select Committee of the Commons," to inquire into the hindrances to the study of anatomy, was appointed April 22, 1828. Its report was rendered on the twenty-second of July, following. In 1788, the Court of King's Bench decided, in the first reported case of the sort, that it was a misdemeanor at common law to carry away a dead body from a church-yard, although for the purpose of dissection, as being an offence *contra bonos mores* and common decency. The Select Committee stated in its report, which was favorable to the petitioners, that, under the law as then interpreted, there was scarcely a student or teacher of anatomy in England who was not indictable for a misdemeanor ; and also that medical men " were liable in a civil action to damages for errors in practice, due to professional ignorance ; though at the same time they might be visited with penalties as criminals for endeavoring to take the only means of obtaining professional knowledge." It was not until the following year, when the complaints of the anatomists and the report of the committee had been emphatically endorsed by the "Burking" horrors of Edinburgh, that leave was obtained, on the fourth of May, to bring in a " Bill, to Prevent the Disinterment of Dead Bodies, and for the Better Regulation of Our Schools of Anatomy."

On the second of November, 1828, it was noised about in Edinburgh that a woman had been murdered on All Hallow Eve for

the sake of her body, which was found in the dissecting room of
Dr. Knox. In the investigation which followed it was discovered
that William Hare, the keeper of a low lodging house in the West
Port, and one of his lodgers, William Burke, had, within less than
a year, committed sixteen murders, and disposed of the bodies of
their victims to the teachers of anatomy. The " Burke " method
was to suffocate the victim, already dead drunk. Throttling was
not resorted to: the nose and mouth were kept tightly closed, and
the smothering was soon effected. It was impossible to connect
Knox with these villians in any way, except as a receiver of stolen
goods for the benefit of the public. Hare turned State's evidence,
but Burke was found guilty, hanged and dissected. His skeleton
adorns the Anatomical Museum of the University of Edinburgh.

The Bill alluded to above was brought into Parliament May 5,
1829, but was thrown out in the House of Lords a month later.
It was not until August 1, 1832, after a long discussion in which
Sir James Mackintosh and Mr. Macaulay took part, that the
" Warburton Bill for Regulating Schools of Anatomy " was enacted.
At this distance in space and time the deliberateness of Parliament
seems a trifle strained in the face of such facts as we have stated ;
but one of the chief glories of the British Constitution is its slow
growth, we believe.

The Warburton Act is, with some trifling amendments, still in
force. Its effect has been to protect the sepulchres of the dead
and, in the long run, to furnish an adequate supply of subjects.
As, however, Massachusetts anticipated Great Britain by more
than a year in legalizing anatomy, in a law based upon the same
principles as those embodied in the English Act, we forego any
special consideration of the terms and provisions of the latter.

PART SECOND.

ANATOMY IN AMERICA.

European and American anatomy have both developed along
the same lines, but the European type is more highly specialized.
Nearly all the developmental stages through which European
anatomical science has passed are to-day represented in various
States of the American Union. In some States it is a secret and
perilous pursuit ; in others it has gained legal protection ; in a few
it has attained, perhaps, to the dignity of an ungenerously fostered
science.

The earliest utterance in America, in recognition of the importance of anatomical studies, seems to have been made in Massachusetts. In "The Cleare Sun-Shine of the Gospel Breaking upon the Indians in New England" is found a letter dated "Roxbury, 24 September 1647," from John Eliot to the Rev. Thomas Shephard of "Cambridge in New England." The Apostle declares of the Indians that "all the refuge they have and relie upon in time of sickness is their Powwaws, who, by antick, foolish and notional conceits delude the poor people, so that it is a very needfull thing to informe them in the use of Physick, and a most effectuall meanes to take them off from their Powwawing. Some of the wiser sort I have stirred up to get this skill; I have showed them the Anatomy of man's body, and some generall principles of Physick. I have had many thoughts in my heart that it were a singular good work, if the Lord would stirre up the hearts of some or other of his people in England to give some maintenance toward some Schoole or Collegiate exercise this way, wherein there should be Anatomies and other instructions that way." It is unlikely that the Apostle Eliot added dissections to his lectures on "the Anatomy of man's body;" for later in the same letter he deplores the fact that "our young students in Physick have onely theoreticall knowledge, and are forced to fall to practice before ever they saw an Anatomy made," and says, "We never had but one Anatomy in the Countrey, which Mr. Giles Firman (now in England) did make and read upon very well."

The "first Anatomy in the Countrey" was doubtless made without the warrant of legal enactment; certainly the majority of dissections since then have been so made. The first statutory provision regarding anatomy in America seems to be the Massachusetts Act of 1784, by the terms of which the bodies of those killed in duels and of those executed for killing another in a duel might be given up to the surgeons "to be dissected and anatomized." In 1831 Massachusetts anticipated all her sister States, and England as well, by legalizing the study of "anatomy in certain cases."

In the Diary of Samuel Sewall, of Boston, recently published by the Massachusetts Historical Society, is found under date of September 22, 1676, the following entry: "Spent the day from 9 in the M. with Mr. [Dr.] Brakenbury, Mr. Thomson, Butler, Hooper, Cragg, Pemberton, dissecting the middle-most of the

Indians executed the day before. X, who taking the ♥ in hand, affirmed it to be the stomach."

The earliest reference that I have found to a post-mortem examination in America is contained in a manuscript order of the Council of Lord Baltimore, dated St. Mary's, in Maryland, July 20, 1670. In it John Stansley and John Peerce, Chyrurgeons, are ordered to view, on Monday, August 8, 1670, the head of one Benjamin Price, supposed to have been killed by the Indians. It was brought out in connection with the Salem witchcraft trials, in 1692, that "about seventeen years before," a jury had been impanelled upon the body of a man that had died suddenly in the house of Giles Corey, and that the jury, among whom was Dr. Zerubbabel Endicott, found the man "bruised to death, and having clodders of blood about the Heart." This would indicate that a post-mortem examination was made in Massachusetts as early as 1675, fifteen years prior to that made on the body of Governor Slaughter, of New York, which is usually cited as the first recorded autopsy in America. In 1690, Governor Slaughter died suddenly, under circumstances which excited suspicions of poisoning. Dr. Johannes Kerfbyle, assisted by five physicians, examined the body. The Council ordered £8 8s. to be paid the surgeons for their examination.

It is recorded that Dr. John Bard and Dr. Peter Middleton, of New York city, in 1750 injected and dissected the body of Hermanus Carroll, an executed criminal, "for the instruction of the young men then engaged in the study of medicine." This was thirty-nine years before the State of New York legalized the dissection of the bodies of malefactors executed for arson, burglary, or murder. Though Pennsylvania passed no anatomy Act until 1867, the first American medical school was organized in Philadelphia in 1765, by Drs. Morgan and Shippen, natives of that city. Dr. William Shippen, Jr., a pupil of John and William Hunter, gave, in 1762, a systematic course of lectures on anatomy. This first course of lectures by Dr. Shippen, is usually termed the first full and scientific course of anatomical lectures given in America ; although Dr. Cadwallader, as early as 1751, made dissections for the benefit of the physicians of Philadelphia, and Thomas Wood, surgeon, in 1752 advertised in the New York papers "a course on osteology and myology in the city of New Brunswick, N. J.," to be followed, in case of proper encouragement, by a course in angi-

ology and neurology, and a course of operations on the dead body.
It should also be noted that Dr. William Hunter, educated at
Edinburgh under the elder Monro, who came to America in 1752,
gave lectures on anatomy and surgery in Newport, R. I., in the
years 1754, 1755, and 1756.

Shippen's courses were so successful that in 1765 the Medical
College of Philadelphia was organized with two professorships.
Dr. Shippen held the chair of "anatomy and surgery;" that of
the "theory and practice of physic" was filled by Dr. John
Morgan.

A brief consideration of the character and career of Dr. William
Shippen, Jr., the first Professor of Anatomy and Surgery in
America, may well detain us for a few moments. His father, Dr.
William Shippen, was an eminent physician in Philadelphia, in
which city the son was born, in 1736. Young Shippen graduated
in 1754, at the College of New Jersey, of which institution his
father was one of the founders. After studying medicine for three
years with his father, he repaired to Europe, where he studied at
Edinburgh and London. He returned to Philadelphia in 1762,
in which year, at the age of twenty-six, he gave his first course of
lectures on anatomy. One of his successors in the chair of anatomy
—Dr. W. E. Horner—says: "Dr. Shippen seems to have been
intended by nature to lay the corner-stone of the immense edifice
of medicine, which has since been erected in this country. Aged
twenty-six, at the period alluded to, uncommonly perfect in his
form and engaging in his aspect; his manners were those of a
finished gentleman; his enunciation was fine; his temper invari-
ably sprightly and good, could neither be excited by rancor, nor
rendered sullen and morose by opposition. To the personal ad-
vantages stated, and those of extensive hereditary friendship and
family alliance, Dr. Shippen added foreign study—at that day all
important in public estimation, from the want of opportunities of
instruction here. While in London he lived in the family of Mr.
John Hunter, the celebrated surgeon, and followed the lectures of
Dr. William Hunter on anatomy and mid-wifery. He enjoyed
the advantages of great intimacy with Sir John Pringle and Dr.
Fothergill. To the incentive of such illustrious associations we
may attribute much of the energy and determination which marked
his subsequent career. Dr. Shippen arrived in Philadelphia in the
Spring of 1762, having completed his studies and gained from his
preceptors the reputation of great natural talents."

In the Pennsylvania *Gazette*, published by B. Franklin, Postmaster, and D. Hall, November 11, 1762, I find a card from Dr. Shippen which, inasmuch as I cannot find that it has been republished, I venture to quote as a whole:

PHILADELPHIA, November 11.

MR. HALL. SIR:

Please to inform the Public that a Course of Anatomical Lectures will be opened this Winter in Philadelphia for the Advantage of the young Gentlemen now engaged in the Study of Physic in this and the neighboring Provinces, whose Circumstances and Connections will not admit of their going abroad for Improvement to the Anatomical Schools of Europe; and also for the entertainment of any Gentlemen who may have the Curiosity to understand the Anatomy of the Human Frame.

In these Lectures the Situation, Figure and Structure of all the parts of the Human Body will be demonstrated; their respective uses explained, and, as far as a Course of Anatomy will permit, their Diseases, with the Indications and Method of Cure, briefly treated of; all the necessary Operations in Surgery will be performed, a Course of Bandages exhibited, and the whole conclude with an Explanation of some of the curious Phenomena that arise from an examination of the Gravid Uterus, and a few plain general Directions in the Study and Practice of Midwifery.

The Necessity and public Utility of such a Course in this growing Country, and the Method to be pursued therein, will be more particularly explained in an Introductory Lecture, to be delivered the 16th Instant, at six o'clock in the Evening, at the State House, by William Shippen, jun., M. D.

N. B. The Managers and Physicians of the Pennsylvania Hospital, at a Special Meeting, have generously consented to countenance and encourage this undertaking; and to make it more entertaining and profitable, have granted him the use of some curious Anatomical Casts and Drawings (just arrived in the Carolina, Capt. Friend) presented by the judicious and benevolent Doctor Fothergill, who has improved every Opportunity of promoting the Interest and Usefulness of that noble and flourishing Institution.

The Pennsylvania *Gazette*, of November 25, 1762, contains the following announcement:

Dr. Shippen's anatomical lectures will begin to-morrow evening at six o'clock, at his father's house, in Fourth street. Tickets for the

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course to be had of the doctor at five pistoles each, and any gentlemen who incline to see the subject prepared for the lectures, and learn the art of dissecting, injections, etc., are to pay five pistoles more.

It is stated that his first class numbered twelve. "Having thus started, it is not to be understood," says Dr. Horner, "that the lectures proceeded without occasional interruptions from popular indignation; for the city being small, almost everyone knew what was going on in it. The house was frequently stoned, and the windows broken; and on one occasion, Dr. Shippen's life was put into imminent danger. While engaged within, the populace assembled tumultuously around the house. His carriage fortunately was at the door, and the people supposing that he was in it made their first attack there. The windows of the carriage being up, they were speedily demolished with stones, and a musket ball was shot through the body of the carriage; the coachman applied the whip to his horses and only saved himself and his vehicle by a rapid retreat under a shower of missiles. The Doctor hearing the uproar, ascertained its cause, and extricated himself through a private alley."

Possibly the riot above described by Dr. Horner, may have elicited the following utterance from Dr. Shippen, which is printed in the Pennsylvania *Gazette*, December 26, 1765:

It has given Dr. Shippen much Pain to hear that notwithstanding all the Caution and Care he has taken to preserve the utmost Decency in opening and dissecting dead Bodies, which he has persevered in chiefly from the Motive of being useful to Mankind, some evil-minded Persons, either wantonly or maliciously, have reported to his Disadvantage that he has taken up some Persons who were buried in the Church Burying Ground, which has disturbed the Minds of some of his worthy Fellow Citizens. The Doctor with much Pleasure, improves this Opportunity to declare that the Report is absolutely false; and to assure them that the Bodies he dissected were either of Persons who had wilfully murdered themselves or were publicly executed, except now and then one from the Potter's Field, whose Death was owing to some particular Disease; and that he never had one Body from the Church.

In Chapter CCXI of the "History of the City of Philadelphia," written by Westcott Thompson, but not yet published in book form, are found the following statements regarding Dr. Shippen:

"Late in November 1762, Dr. Shippen received the first subject for dissection of which there is any record. A negro man having cut his throat with a glass bottle, from the effect of which he died, the action upon his case is thus recorded by the *Gazette* of December 2. 'After the coroner's jury had pronounced him guilty of self murder, his body was immediately ordered *by authority* to Dr. Shippen's anatomical theatre,' this accession to the stock of the dissecting room must have been received a day or two after the opening lecture.

"In September 1765, Dr. Shippen was compelled to deny publicly that he had taken dead bodies for the purposes of dissection from the church burying grounds. In September 1768, he was again obliged to contradict the rumor that he had taken dead bodies from the city burying grounds for purposes of dissection. In 1770, considerable excitement existed in the city in relation to the supposed removal of dead bodies from the city burying grounds for dissection in the anatomical department of the college. It was probably about that time that the circumstances happened described by Dr. Carson. [History of the Medical Department of the University of Pennsylvania, pp. 81 and 217.] 'On one occasion Dr. Shippen's house was mobbed and only by exercising great tact and by the judicious interference of his friends, and of the authorities, was he saved from the entire destruction of his accumulated materials for teaching. This event was known for years as the sailors' mob.'

"Dr. Shippen in *Bradford's Journal* of January 11, 1770, published an address to the public in which he said there were wicked and malicious reports of his taking up bodies from several burying grounds. He said 'I declare that I never have had, and that I never will have directly or indirectly, any subject from any burying ground of any Christian denomination whatever.' He said that upon two of the families terrified by this report, he had waited, in order to vindicate himself. He had tried to trace out the authors of the reports but had failed. It was generally believed that he had taken up the body of a young lady from Christ Church burying ground, 'but within a few days the grave had been opened, and the body found there.' Another body was that of a woman, whose name is given by Dr. Shippen. He says that 'she died in the middle of the summer of 1769, of a putrid fever, and yet I am charged with dissecting her body in the middle of winter.' In corroboration of this address he appended an affidavit by Joseph Harrison who stated that he was a student of medicine and had lived with Dr. Shippen, Sr., as an apprentice, 'for the last eight years;' that he had regularly attended the courses of Dr. Shippen, Jr., and knew where the subjects employed in his lectures were from. He said, 'none

were ever taken out of any burying ground of a Religious Society in this city.' "

When Dr. Shippen's lectures were interrupted, in 1775, by the breaking out of the Revolution, his class numbered between thirty and forty students. Early in 1777, he was appointed Medical Director General of the Continental army. In 1778, he resumed his lectures in Philadelphia. In 1781, he resigned his position in the army to devote himself to the medical school. Dr. Caspar Wistar became Shippen's associate in 1792. Dr. Shippen died in 1808.

In New York and Massachusetts, as in Pennsylvania, the anatomists were the founders of the first medical schools. The medical department of King's, now Columbia College, was organized in New York, in 1767. Dr. Samuel Clossy, an Irishman, who began his course of lectures on anatomy in New York in 1763, was chosen the first professor of anatomy in King's. Dr. John Warren, who from 1777, till the close of the Revolution, had served as surgeon-in-chief of the military hospitals at Boston, gave a private course of dissections to a class of medical students in that city in 1780. In the following year he gave a public course of anatomical lectures, the success of which led to the organization of the Harvard Medical School in 1782. Dr. Warren was the first professor in the new school. He was for many years its presiding genius, and held the professorship of anatomy and surgery till his death in 1815. It was chiefly through the efforts of Dr. Nathan Smith, that the Dartmouth Medical School was founded, in 1797. Dr. Smith was appointed "to deliver public lectures upon Anatomy, Surgery, Chemistry, Materia Medica, and the Theory and Practice of Physic." To the Dartmouth School is usually assigned the fourth and final place on the list of American schools of medicine founded before 1800.

Thanks to the efforts of Thomas Jefferson, in 1779, Virginia can claim a place on that list for the medical department of William and Mary College. "I effected in that year, 1779," he says in his autobiography, "a change in the organization of that institution by abolishing the Grammar school and the two professorships of Divinity and Oriental languages, and substituting a professorship of Law and Police, one of Anatomy, Medicine, and Chemistry, and one of Modern Languages." In 1778, Mr. Jefferson drew

up a "Bill proportioning Crimes and punishments in Cases heretofore capital." Among its provisions was the following: "If any person commit petty treason, or a husband murder his wife, a parent his child, or a child his parent, he shall suffer death by hanging, and his body be delivered to Anatomists to be dissected." This bill was lost by the majority of a single vote, and Virginia lost the opportunity of passing the first American Act to legalize anatomy in even a small way. Virginia as yet has no anatomy act.

In December, 1692, the province of Massachusetts Bay incorporated the major portion of the English Act of 1604 against witchcraft among its statutes. The history and provisions of this Act are worthy of more than passing mention, because it contains not only the first American, but also the first English, statutory prohibition of the desecration of graves, and indicates full well that the belief in sorcery was a potent factor in popular prejudice against human dissections. In the preamble to an Act for "the appointing of Physicians and Surgeons," passed in 3 Henry VIII., 1511, it is recited that "so far forth were the Science and Cunning of Physick and Surgery practised by ignorant persons, that common Artificers, as Smiths, Weavers, and Women, boldly and accustomably took upon themselves great cures, and things of great Difficulty, in the which they partly use Sorcery and Witchcraft, partly apply such medicines unto the Disease as be very noious and nothing meet therefor." The practice of witchcraft was first made a felony, punishable with death and the forfeiture of estate to the King, in 1541. This Act of the Parliament of 33 Henry VIII. was repealed six years later, in the first year of Edward VI.; but in 1565, the fifth year of Queen Elizabeth, it was reënacted with a saving clause, whereby dower was secured to the widow and inheritance to the heir of the felon. In 1604, the first year of James I., the Act of 5 Elizabeth, as well as that of the 9th Parliament of Mary of Scotland, was repealed, and an Act for "the better restraining and more severe punishing of witchcraft and dealing with evil and wicked spirits," was passed. It contained the following provision, new to the English law: "If any person shall take up any dead man, woman, or child out of his, her, or their grave, or any other place where the dead body resteth, or the skin, bone, or any other part of any dead person, to be employed in any manner of witchcraft, inchantment, charm, or

sorcery, whereby any person shall be killed, destroyed, wasted, consumed, pined, or lamed in his or her body, or any part thereof," every such offender "shall suffer pains of death as a felon, and shall lose the benefit of clergy and sanctuary."

This Act was cited formally in indictments drawn in Maryland in 1674, and in Massachusetts in the spring of 1692, and was acknowledged to be in full force in Pennsylvania in 1684. Massachusetts seems to have been the only colony to embody it in its laws. The Privy Council repealed the Act in 1695, because it was "not found to agree with ye Statute of King James the First whereby ye Dower is saved to ye Widow and ye Inheritance to ye heir of ye party convicted." The English Act remained unrepealed till 1736; and, so late as 1712, was declared to be in force in South Carolina. It does not appear that any "resurrectionist" was ever convicted under it in America. The first American Act to prevent the digging up of bodies for dissection, was the New York Act of 1789.

As we have already seen, Pennsylvania, New York, Virginia, Massachusetts and New Hampshire all had medical schools previously to 1800. As late as 1782, when the Harvard Medical School was organized, no one of the above-mentioned States had a law in its statute books touching the dissection of the dead or the desecration of their graves. The utmost help given to anatomists was the occasional allowance of the body of a suicide or executed criminal.

Possibly, Governor John Winthrop, who was read in physic, may have authorized his kinsman, Giles Firmin, to make the anatomy mentioned by Eliot. Prior to the Revolution, the royal governors could order the dissection of a murderer's body. In 1778 the State of Virginia refused to sanction the dissection of executed murderers; and has apparently remained in a state of arrested development ever since, so far as any appreciation of the claims of anatomy is concerned. Massachusetts, in 1784, passed a law allowing the dissection of dead duelists, thereby unwittingly reproducing in spirit, though not in letter, a canon of the mediæval church, which denied Christian burial to men slain in tournaments. New York, in 1789, in order that science might not be injured by its law of that year regarding disinterment, made it lawful for the courts to add dissection to the death penalty in cases of murder, arson and burglary. The First Congress of the United States, by

the act of April 30, 1790, gave federal judges the discretion of adding dissection to the sentence of convicted murderers. A similar act was passed by New Jersey in 1796. No trace of progress, worth mentioning, in this class of legislation, since the enactments noted, is to be found in the most recent revisions of statutes, either of the United States or of New Jersey.

The Act of Massachusetts, passed in 1784, against duelling, is a noteworthy one, by reason of the fact that it contains the first authorization on the part of an American legislature of the dissection of the dead bodies of malefactors. The province had enacted laws for the prevention of duelling, in 1719 and 1729. That of 1719 provided penalties in the way of fine, imprisonment, and corporal punishment—any or all of them, at the court's discretion —for those convicted of engaging in, or challenging another to engage in, a duel. Under the Act of 1729, duellists and their accomplices were carried in a cart to the gallows with a rope about the neck, "and after sitting for the space of one hour on the gallows, with the rope about his neck as aforesaid," the offender was confined in the common jail for one year, and at the expiration of his sentence was required to find sureties for his good behavior for the succeeding twelvemonth. The Acts of 1729 and 1784, both denied Christian burial to the bodies of men killed in a duel. Moreover, it was provided in section 3 of the Act of 1784, "that when it shall appear by the coroner's inquest that any person hath been killed in fighting a duel, the coroner of the county where the fact was committed shall be directed and empowered to take effectual care that the body of such person so killed be immediately secured and buried without a coffin, with a stake drove through the body, at or near the usual place of execution, or shall deliver the body to any surgeon or surgeons, to be dissected or anatomized, that shall request the same and engage to apply the body to that use." Section 4 ordains "that any person who shall slay or kill another in a duel, and shall, upon conviction thereof on an indictment for murder, receive sentence of death, part of the judgment of the court upon such conviction shall be that the body be delivered to any surgeon or surgeons, to be dissected and anatomized, that shall appear in a reasonable time after execution to take the body and engage to apply it to that purpose."

If the Massachusetts legislators in 1784 had any intention of recognizing the needs of the anatomists, they failed to declare it, so that New York was the first State, by section 2 of its Act of 1789, to express the desire that "science might not in this respect be injured by preventing the dissection of proper subjects." It was not till the passage of the Massachusetts Act of 1831 that any State really undertook to "legalize the study of anatomy."

It is most likely that the provisions of the Act of 1784 touching dissection were designed to make duelling a specially infamous offence. This was quite in keeping with the English law regarding dissection. In 1752, the Parliament of 22 George II., in order that "some further Terror and peculiar Mark of Infamy might be added to the Punishment of Death," legalized the delivery of the bodies of executed murderers to the Surgeons for dissection. This must have been the Act from which the royal governors derived authority to dispose of murderers' bodies in Massachusetts in the manner indicated in the following extract, taken from the Life of Dr. John Warren, by Edward Warren, M. D., page 230: "At this period [just prior to the Revolution] the governor had the disposal of the body of the criminal after execution. He might order its delivery to the man's friends, to any one to whom he himself assigned it, or to a surgeon. The prisoner, with the governor's assent, might make his own arrangements even for the sale of his body, if he was so disposed, either for the benefit of his family or his own brief enjoyment."

It is to be remarked that the Act of 1752 required the judges to add either dissection or hanging in chains to the death sentence of murderers, and that previously to 1832, when the Warburton Anatomy Bill was passed, there seems to have been no warrant in English law for any sort of bargain concerning a cadaver. The only legal mode of disposing of a dead body, excepting in case of malefactors, was to bury it. Once buried, it was an indictable offence at common law for any person to exhume it, except by the leave of the proper officers.

The New York Act of 1789 is of especial interest; both on account of the circumstances which led to its enactment and because it may fairly be considered the germ of all subsequent American legislation concerning the cadaver. The Act of 1789 seems to have owed its existence to the Doctors' Riot, in New York City, in April, 1788. If you will turn to the issue of the *New York*

Journal and Patriotic Daily Register, for Tuesday, April 15, 1788, you will find the following: " As a concise statement of the sad confusion of the city since Sunday last could not be ascertained for this day's paper, it was thought proper to postpone it till such an one could be had. It is devoutly to be hoped, in the meantime, that those who feel themselves injured by the DOCTORS will SERIOUSLY REFLECT upon the fatal ERROR of revenging their cause upon the public at large." Imagine the *New York Herald* apologizing for its inability to give a concise statement concerning a riot two days old ! On Wednesday the *Register* avows a peculiar satisfaction in announcing " that the unhappy convulsions of this city have very considerably subsided," and promises " some particulars respecting this melancholy transaction from peace to horrid war in the *Weekly Register* to-morrow." The charge of "his Honor Chief-Justice Morris," delivered the day previous to the grand jury at the City Hall, is contained in the *Weekly Register* of Thursday, April 17, 1788; but one looks in vain for the promised particulars in that or the succeeding issues of the *Daily Patriotic Register.* The affidavit of Dr. Richard Bayley, executed April 14, is found in the *Register* of the following day. In it he denies any agency or concern " in removing the bodies of any person or persons, interred in any church-yard or cemetery, belonging to any place of public worship, and that he hath not offered any sum of money to procure any human body so interred, for the purposes of dissection," and further saith, " that no person or persons under his tuition have had any agency or concern in digging up or removing any dead body interred in any of the church-yards or cemeteries, to his knowledge or belief, and further this deponent saith not." Similar affidavits on the part of Ebenezer Graham, John Parker, and George Gillaspy, pupils of Dr. Charles McKnight, professor of anatomy ; also of Dr. McKnight himself, and of John Hicks, Sen., are to be found in the *Weekly Register,* which contains not only the Chief-Justice's charge, already mentioned, but also the card of William Neilson, Foreman of the Grand Jury. The grand jury " do request that those persons who can give any information that may lead to a discovery will acquaint them therewith during their present sitting, at Simmons' Tavern, in Wall Street." We find the local news of the New York of a hundred years since best reported in the New York letters of the Boston and Philadelphia papers. The *Boston Gazette*

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and the Country Journal, in its issues of April 28 and May 5, 1788, contains full accounts of the New York mob. The first account is from a letter written April 16, by one who had borne arms against the rioters. I give the second, both because it is shorter, and because its writer seems to have taken especial pains to be accurate.

NEW YORK, April 25th.

As exaggerated accounts of the late riots in this city have been circulated through different parts of the country, we have obtained the following particulars of that unhappy event: During the last Winter, some students of physic and other persons had dug up from several of the cemeteries in this city a number of dead bodies for dissection. This practice had been conducted in so indecent a manner that it raised a considerable clamor among the people. The interments not only of strangers and the blacks had been disturbed, but the corpses of some respectable persons had been removed. These circumstances most sensibly agitated the feelings of the friends of the deceased, and wrought up the passions of the populace to a ferment.

On Sunday, the 13th instant, a number of boys, we are informed, who were playing in the rear of the hospital, perceived a limb which was imprudently hung out of the window to dry; they immediately informed some persons; a multitude soon collected, entered the hospital, and, in their fury, destroyed a number of anatomical preparations, some of which, we are told, were imported from foreign countries; one or two fresh subjects were found, all of which were interred the same evening. Several young doctors narrowly escaped the fury of the people, and would inevitably have suffered very seriously had not His Honor the Mayor, the Sheriff and some other persons interfered and rescued them by lodging them in a gaol. The friends of good order hoped that the affair would have ended here; but they were unhappily mistaken. On Monday morning a number of people collected, and were determined to search the houses of the suspected physicians. His Excellency the Governor, His Honor the Chancellor, and His Worship the Mayor, finding that the passions of the people were irritated, went among them and endeavored to dissuade them from committing unnecessary depredations. They addressed the people pathetically and promised them every satisfaction which the laws of the country can give. This had considerable effect upon many, who, after examining the houses of the suspected doctors, retired to their homes. But in the afternoon the affair assumed a different aspect. A mob, more fond of riot and confusion than a reliance upon the promises of the magistrates and obedience to the laws, went to the gaol and demanded the doctors

who were there imprisoned. The magistrates finding that the mild language of persuasion was of no avail, were obliged to order out the militia to suppress the riot, to maintain the dignity of the government, and protect the gaol. A small party of about eighteen armed men assembled at 3 o'clock and marched thither. The mob permitted them to pass through with no other insult than a few volleys of stones, dirt, etc. Another party of about twelve men, about an hour afterward, made a similar attempt, but having no order to resist, the mob surrounded them, seized and destroyed their arms. This gave the mobility fresh courage; they then endeavored to force the gaol; but were repulsed by a handful of men, who barely sustained an attack of several hours. They then destroyed the windows of that building with stones, and tore down part of the fence. At dusk another party of armed citizens marched to the relief of the gaol, and, as they approached it, the mob huzzaing began a heavy fire with brickbats, etc. Several of this party were much hurt, and in their own defence were obliged to fire; upon which three or four persons were killed and a number wounded. The mob shortly after dispersed. On Tuesday morning the militia of General Malcom's brigade and Col. Bauman's regiment of artillery were ordered out, and a detachment from each were under arms during that day and the subsequent night. But happily the mob did not again collect, and the peace of the city is once more restored.

Dr. J. M. Toner, of Washington, in his useful Annals of Medical Progress, states, p. 97, that "the doctors' mob in 1788, marked the last serious resistance of the populace to the teaching of practical anatomy in America." The very next year, however, as we learn from Griffiths' "Annals of Baltimore," the body of "one Cassidy, lately executed, was obtained for dissection, but was discovered by the populace and taken from the gentlemen who were then studying anatomy and surgery." Dr. Nathaniel Potter, in a pamphlet published in 1838, entitled "Some Account of the Rise and Progress of the University of Maryland," alludes to the destruction in 1807 of the Anatomical Theatre of Dr. Jno. B. Davidge, then a private teacher of anatomy and surgery. Dr. Davidge had erected a small anatomical theatre, at his own expense and on his own ground. "It was discovered by the populace that he had introduced a subject for dissection; the assemblage of a few boys before the door was soon accumulated into a thickly embodied mob, which demolished the house and put a period to all further proceedings for that season." "Such were the vulgar prejudices against dissections," he adds, "that

little sympathy was felt for the doctor's loss." I have been told that a somewhat similar riot occurred in New Haven about the year 1820; I have not been able to verify the statement, however.

The name of Warren is most intimately associated with the rise and progress of anatomical science in Massachusetts. Dr. John Warren while a student in Harvard College, where he graduated in 1771, was the leading spirit in forming a private anatomical society, composed of students. He says of it that "brutes were dissected and demonstrations on the bones of the human skeleton were delivered by the members." The Anatomical Society and the Spunker Club, to which there are frequent allusions in the Life of Dr. John Warren, seem to have been identical. Dr. Warren was the principal lecturer of the club. His most zealous associates were his classmates, Jonathan Norwood, William Eustis, class of 1772, and David Townsend and Samuel Adams, of the class of 1770. Adams was a son of Samuel Adams the patriot. Eustis, Adams, and Warren all studied medicine with an elder brother of the latter, Dr. and General Joseph Warren. Eustis, Warren, Townsend, and Adams became surgeons in the Continental army. Adams died in 1778. Eustis, lived to become governor of Massachusetts in 1823. Warren was surgeon-general of the military hospital at Boston, from June, 1777, till the close of the Revolution, and was the first professor of anatomy and surgery of the Harvard Medical School, of which he was practically the founder.

Some notion of the methods of study of the Spunker Club may be gained from the following extracts from letters written by Eustis to Warren, prior to 1775: "This may serve to inform you that as soon as the body of Levi Ames was pronounced dead, by Dr. Jeffries, it was delivered by the sheriff to a person who carried it in a cart to the water side, where it was received into a boat filled with about twelve of Stillman's crew, who rowed it over to Dorchester Point. . . . . . When we saw the boat at Dorchester Point, we had a consultation, and Norwood, David, One Allen and myself took chaise and rode round to the Point, Spunkers like; but the many obstacles we had to encounter, made it eleven o'clock before we reached the Point, where we searched and searched, and rid, hunted, and waded, but, alas, in vain! There was no corpse to be found. . . . . . We have a —— from another place, so Church shan't be disappointed. P. S. By the way, we have since heard that Stillman's gang rowed him back from the Point up to

the town, and after laying him out in mode and figure buried him, God knows where! Clark & Co. went to the Point to look for him, but were disappointed, as well as we." No wonder that the same writer, in another letter, says, "Good heavens! to reflect on the continued bars we are meeting in our pursuits! It seems as if fate had placed medical knowledge *profunda in puteo, saxis et vix mobilibus submersa.*"

It is not yet one hundred years since Dr. John Warren delivered the first course of public anatomical lectures ever given in Massachusetts, in compliance with a vote of invitation passed by the Boston Medical Society, November 3, 1781. It is scarcely fifty years since the Massachusetts Medical Society began to agitate the question of legalizing the study of anatomy. The Harvard Medical School, in the ninety-eight years of its history, has had but three professors of anatomy, namely, Dr. John Warren, professor of anatomy and surgery from 1782 till 1815, when he died; Dr. John C. Warren, professor of anatomy and surgery from 1815 to 1847, when he resigned; and Dr. Oliver Wendell Holmes, professor of anatomy, who, like the elder Warren, has held his chair thirty-three years.

Dr. John Warren's son and successor, Dr. John C. Warren, was three years old in 1781, the year the Massachusetts Medical Society was incorporated. Fifty years later, as one of the most prominent members of that society, February 2, 1831, he lectured before the members of the Massachusetts Legislature, in the representatives' chamber, on the Study of Anatomy, in accordance with a vote of the house of representatives, passed January 29, 1831. At the time of this lecture the anatomy bill, which became a law on the 28th of that month, was still pending.

No better testimony concerning the obstacles which beset the pursuit of anatomical science during those fifty years can be given than is found in the Biographical Notes of Dr. John C. Warren, from which we quote: "No occurrences in the course of my life have given me more trouble and anxiety than the procuring of subjects for dissection. My father began to dissect early in the Revolutionary War. He obtained the office of army-surgeon when the Revolution broke out, and was able to procure a multitude of subjects from having access to the bodies of soldiers who had died without relations. In consequence of these opportunities he began to lecture on anatomy in 1781. After the peace there was great

difficulty in getting subjects. Bodies of executed criminals were occasionally procured, and sometimes a pauper subject was obtained, averaging not more than two a year. While in college I began the business of getting subjects in 1796. Having understood that a man without relations was to be buried in the North Burying-Ground, I formed a party. . . . . . When my father came up in the morning to lecture, and found that I had been engaged in this scrape, he was very much alarmed, but when the body was uncovered, and he saw what a fine, healthy subject it was, he seemed to be as much pleased as I ever saw him. This body lasted the course through. Things went on this way till 1807, when, with the coöperation of my father, I opened a dissecting-room at 49 Marlborough Street. Here, by the aid of students, a large supply of bodies was obtained for some years, affording abundant means of dissection to physicians and students. In the meantime, however, schools began to be formed in other parts of New England, and students were sent to Boston to procure subjects. The exhumations were conducted in a careless way. Thus the suspicion of the police was excited; they were directed to employ all the preventive measures possible, and watches were set in the burying-grounds. Thus the procuring of bodies was very much diminished, and we were obliged to resort to the most dangerous expedients, and, finally, to the city of New York, at a great expense of money and great hazard of being discovered. Two or three times our agents were actually seized by the police, and recognized to appear in court. One or two were brought in guilty, and punished by fine, but the law officers, being more liberal in their views than the city officers, made the penalty as small as possible. Constant efforts were necessary to carry on this business, and every species of danger was involved in its prosecution. . . . . . At that time scarcely any exhumation occurred without accidents of the most disagreeable and sometimes painful character. The record of them would make a black-book, which, though the odium of it should belong to few individuals, would do no credit to the enlightenment of Boston in the nineteenth century, and convey an idea of the state of feeling of a professor of anatomy on the approach and during the course of his anatomical pursuits.

"Sometimes popular excitement was got up, and the medical college threatened. I had reasons, at some periods, even to

apprehend attacks on my dwelling-house. Whenever the lectures approached, a state of incessant anxiety came with them. At length the pressure was so great that it was resolved to make an effort in the legislature, though with little hope of success."

If it were necessary, evidence to corroborate that of Dr. Warren might be indefinitely multiplied from the published and unpublished traditions of the elders. We content ourselves with the mention of one episode. About 1820 a highly respectable physician of Eastern Massachusetts, being detected in anatomical pursuits, was obliged to flee the State. In a distant community, which to this day has no anatomy Act, he won eminence as a teacher of anatomy and practitioner of medicine.

Dr. H. I. Bowditch, in his Life of Amos Twitchell, M. D., treats fully of the condition of affairs in New England, when the law said, as he puts it, " A man who is found with a body in his possession for the purpose of dissection shall be considered guilty of a felony."

It was chiefly due to the efforts of the Massachusetts Medical Society that Massachusetts, in 1831, was induced to anticipate all English-speaking States in the enactment of a liberal law regarding anatomical science. The first definite action of the society seems to have been taken by the councillors February 4, 1829, when, on the motion of Dr. A. L. Peirson, of Salem, a committee, consisting of Drs. John C. Warren, E. Alden and A. L. Peirson, was appointed " to prepare a petition to the legislature to modify the existing laws which now operate to prohibit the procuring of subjects for anatomical dissections." Previous attempts, however, seem to have been made to weaken popular and legislative prejudices. Public attention had been forcibly called as early as 1820, in the case of the physician above alluded to, to the unsatisfactory working of the law of 1815, " to protect the sepulchres of the dead." It is said that a year or two later a private teacher of anatomy, in Boston, found one morning on his dissecting-table the body of a prominent actor, then recently deceased. The anatomist, who had been a particular admirer and friend of the actor's, caused the body to be returned to the tomb, under Trinity Church, from which it had been stolen, and acquainted the authorities with the circumstance. This occurrence seems never to have been made public, but the physicians and authorities agreed that the laws must be amended. Doubtless they concluded that the

public must be enlightened before anything could be gained from the legislature, for, in 1825, Wells and Lilly reprinted in pamphlet form an article on "The Importance of the Study of Anatomy, with some Additional Remarks," from the *Westminster Review* of 1824. Some writers allude to efforts before the legislature in 1828, but we have found no documentary proof of any legislative action previous to that in the house of representatives, February 3, 1829, when the Committee on the Judiciary was instructed, on motion of Mr. F. A. Packard of Springfield, "to inquire into the expediency of making any farther legal provisions to protect the sepulchres of the dead from violation." In accordance with these instructions, on February 14th the Committee reported a bill, which, on being read a second time, February 24th, was indefinitely postponed on the motion of Mr. Thomas B. Strong, of Pittsfield. The secretary of the Massachusetts Medical Society at this time was Dr. George Hayward. In the *North American Review* for January, 1831, he says that this proposition, above noted, to mitigate the severity of the law "was hardly listened to with decency; members seemed anxious to outdo each other in expressions of abhorrence; and the bill was not even allowed a second reading."

History repeats itself in the case of anatomy Acts no less than in other departments. In 1866, an anatomy bill, after passing the Pennsylvania house of representatives, was withdrawn from the senate of that State, because a too influential member of that body objected to it as being "unworthy of the age in which we live." The next year, however, when it was made manifest that "the bodies of distinguished legislators themselves, after a life full of good works, were no longer safe in their graves," both senate and house passed "An Act for the promotion of medical science, and to prevent the traffic in human bodies, in the city of Philadelphia and the county of Allegheny."

At the annual meeting of the Fellows of the Massachusetts Medical Society, June 3, 1829, the committee of three, appointed by the councillors in February, reported that it was inexpedient to act upon the petition prepared by them to be presented to the legislature. After a full discussion of the report it was agreed to refer the whole subject to a committee of nine. The committee was requested to report at the October meeting of the councillors; and the councillors were authorized to take such measures as they might deem necessary in behalf of the society. The following

named gentlemen were chosen to serve on this committee: Drs.
A. L. Peirson, of Salem; John C. Warren, John D. Wells, John
Ware, William Ingalls, and George C. Shattuck, of Boston;
Nathaniel Miller, of Franklin; Nehemiah Cutter, of Pepperell,
and John Brooks of Bernardston. When the councillors of the
society met, October 7th, the committee reported that on September
1st a circular letter to the Fellows of the society had been issued,
"with a view of advancing the objects proposed by their appoint-
ment," and they recommended to the councillors to cause a petition
to be prepared and presented at the winter session of the general
court. It was voted to continue the committee, and to authorize
it to incur an expense not exceeding one hundred and fifty dollars.

A circular letter, dated Salem, September 1, 1829, and signed
by all of the committee excepting Dr. Miller and Dr. Cutter,
solicits the aid of every influential member of the society in remov-
ing the popular prejudice against dissection, "especially as it
exists in the minds of members of the legislature." The points
upon which it was intended to rely in the proposed petition to the
legislature, are as follows: "(1.) Anatomical knowledge is abso-
lutely necessary in all branches of our profession. (2.) This
knowledge can only be acquired by dissection. (3.) So far as the
poor are concerned, it is for their especial benefit that all physicians
should learn anatomy thoroughly. (4.) It is believed that the
diseases and lameness of many paupers have passed from a curable
to an incurable condition for the lack of surgical skill, which could
only have been derived from a knowledge of practical anatomy.
(5.) All lovers of good morals must feel desirous to prevent the
growth of a body of people who make it a business to violate the
sepulchres of the dead. (6.) The public, as a body, have a greater
degree of interest in this matter than even physicians." The
Fellows are urged to lay the subject before the members of the
legislature, with whom they may be acquainted, and to inform the
committee, before October 1st, concerning their own views and the
course of public opinion in their vicinity.

The petition authorized by the councillors, and alluded to by
the committee in the circular, which was probably written by
Dr. Peirson, seems to have taken the shape of an "Address to the
Community on the necessity of legalizing the Study of Anatomy :
By order of the Massachusetts Medical Society." In the address,
which covers twenty-seven pages, and bears the imprint of Per-

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kins & Marvin, Boston, 1829, the points of the Salem circular are
amplified and enforced. The address is noticed in the *American
Journal of Medical Sciences*, vol. vi, p. 210, by Dr. W. E. Horner,
of Philadelphia, who characterizes it as " a candid and open expo-
sition of difficulties, and of the means of relieving them." " It
is," he says, "a statement directly to the point, and must have
weight, if common sense and common philanthrophy are to be
arbiters. It proposes that the legal restrictions upon dissections
shall not apply in the case of individuals who have no living
relatives, and who have been kept at the public expense." Dr.
George Hayward declares that "this address made a deep impres-
sion on the thinking part of society, and wrought a marvellous
change in public opinion." At their meeting, on February 3,
1830, the councillors of the Medical Society authorized the com-
mittee of nine to print a new edition of not more than ten thousand
copies of the Address to the Community.

Meanwhile, on January 22d, in accordance with a motion made
by Mr. Mason, of Boston, in the house of representatives, the
Committee on the Judiciary had been instructed to inquire into the
expediency of farther legislation for the protection of sepulchres.
The Judiciary Committee consisted of Messrs. L. Saltonstall, of
Salem; L. Shaw, of Boston; Newton, of Worcester; Mann, of
Dedham; and Whitman, of Pembroke. Mr. Saltonstall, the
chairman, made a detailed report February 25, 1830, in which it
was recommended that the farther consideration of the matter be
referred to the first session of the next legislature. The report
lay upon the table till March 11th, when it was taken up, accepted
and ordered to be published in the " newspapers which print the
laws of the commonwealth." This report is printed as " No. 51,
House Documents, pp. 756–764, Documents of Massachusetts,
Political Year 1829, and January Session 1830." The report is
eminently liberal in spirit, and judicial in tone, and is written
clearly and concisely. Although the committee reach the conclu-
sion that the existing law, that of 1815, is unfair to the medical
profession and inconsistent with the best interests of the commu-
nity, they refrain from proposing any alteration of it, believing
that public opinion has not become sufficiently enlightened to
warrant such action.

Governor Levi Lincoln, in his address to the legislature, deliv-
ered May 29, 1830, at the opening of the summer session, declares

that the frank and manly representation by the medical faculty of
the embarrassments and difficulties of acquiring a knowledge of
anatomy deserves the most respectful regard. "It may be," he
says, "that this subject is of a nature too delicate for direct legis-
lation; but the public mind should be instructed in its interesting
importance. Let it be shown that the knowledge which is sought
in the science of anatomy concerns all the living, and that without
it the accidents and ills of life which art might remedy are beyond
relief. Let the reason of men be addressed, and prejudice be
dispelled by information and the force of argument. It may then
come to be understood that a community which demands the
exercise of skill and denies the means to acquire it, which punishes
ignorance and precludes the possibility of removing it, is scarcely
more compassionate than that Egyptian harshness which imposed
the impracticable task in cruel oppression of the inability to per-
form it. . . . . . It is not my purpose to propose any definite act
for your adoption. I would commend the subject only to the
discreetness of your counsels."

On May 31st, Mr. John Brazer Davis, of Boston, moved in the
house of representatives, and it was ordered, "That so much of his
Excellency the Governor's speech, as relates to a modification of
the laws in relation to the study of anatomy, be referred to a select
committee." The gentlemen chosen to act as such committee, were
Messrs. J. B. Davis, of Boston; G. Willard, of Uxbridge; A.
Hutchinson, of Pepperell; L. W. Humphreys, of Southwick, and
J. B. Flint, of Boston. The day after their appointment, the
committee reported through Mr. Davis, that the subject be referred
to the next session of the legislature, and the report was accepted.

On the 1st of January, 1831, the select committee made its
report, and brought in a bill "more effectually to protect the
sepulchres of the dead and to legalize the study of anatomy in
certain cases." The report was written by the chairman of the
committee, Mr. Davis. The report constitutes No. 4 of the House
Documents for 1831, and in the printed copy is dated January 6.
Pages 3–82 inclusive are devoted to the report proper; the bill is
found on pages 83–86; the list of documents accompanying the
report is found on page 87; and the documents themselves fill
twenty-nine pages more.

This is altogether the most exhaustive document on the subject
that we have seen; inasmuch as the committee undertakes to con-

sider, in "all its aspects, the subject committed to them, and to present not only the results, but the details, of their researches and reasonings on it." We shall not undertake to outline it within the limits of a latter-day paper, in face of the fact that twenty pages octavo are taken up in tracing " the progress of anatomical science from the first rude attempts of the Greeks, through a slow progress of near two thousand years," before it is attempted to show, in nearly thirty-six pages more, that "the study and knowledge of anatomy are essential to the safe and successful practice of medicine." We unhesitatingly recommend this "faithful compilation of the facts and reasonings of distinguished men, who have devoted their attention to this subject," to the consideration of those who have to snatch time from the practice of medicine to get up "inaugural addresses" for medical colleges in States still fifty years behind the times. They will find Dr. Southwood Smith's "Uses of the Dead to the Living," and the "Report of a Select Committee of Parliament on the Hindrances to the Study of Anatomy, London, 1828," poor beside, and because of, the riches of this report of the Davis committee.

The legal status of dissection is noticed in the report as follows: "In 1815 a law was passed for the protection of the sepulchres of the dead, which punished the exhumation of any dead body or the knowingly and wilfully receiving, concealing, or disposing of any such dead body, by a fine of not more than one thousand dollars, or imprisonment for not more than one year. Before the passing of this Act, several cases at common law were brought before the Supreme Judicial Court, in all of which, where there was a conviction, the party was punished. Where it appeared that the exhumation was for subjects for dissection, a small fine was imposed. The last case of this kind was against a now eminent physician, then of Essex county, in which several important law points were raised; but the case does not appear to have been reported. Under the statute there have been several prosecutions, convictions, and punishments. With truth it may be said that in Massachusetts a student or teacher of anatomy cannot be found who is not indictable under the statute of 1815."

"While the law of this Commonwealth is thus severe against the exhumation of dead bodies, another law has been passed, by which every practitioner of medicine is required to obtain a degree at Harvard University, or license from the Medical Society, before

he can maintain an action for debt for his professional services. The license or degree is given on examination, and one of the prerequisites for this examination is that the applicant shall have gone through such a course of dissection as shall give him a minute knowledge of anatomy.

"The only legalized mode of supplying subjects for dissection is the sentence or order of the Supreme Judicial Court of this State and of the Circuit Court of the United States in capital convictions within their respective jurisdictions. The insufficiency of this supply may be inferred from the statements of the secretary of the Commonwealth and of the clerk of the United States District Court. The former states, in answer to inquiries addressed him by the chairman of this committee, that the whole number of executions or suicides of convicts from January 1, 1800, to December 31, 1830, is but twenty-six—less than one a year. The clerk of the United States District Court, in reply to like inquiries, states that from the adoption of the federal constitution and the first organization of the federal courts down to the present time the whole number of executions and of suicides of convicts sentenced by that court in this district is but fourteen,—being about one in three years."

February 26, the clerks of the two houses caused the enacted bill to be laid before Governor Lincoln, by whom it was approved and signed February 28, 1831.

The wisdom of the Medical Society and the select committee in acting on Governor Lincoln's recommendation that "the reason of men be addressed, and prejudice be dispelled by information and the force of argument, is justified by the lack of opposition to the enactment of the Davis bill. The Boston *Advertiser* for February 11, 1831, notes the fact that on the day previous the Davis bill had passed to a third reading in the house by a vote almost unanimous. It adds : "No discussion took place touching the general provisions or tendency of the bill. Several amendments were offered relating to the details only. No one expressed any sentiments or opinions in opposition to the general features of the bill; but it received the approbation of all as a necessary step in the progress of improvement." This shows a marked change in public opinion since 1829, "when," to use the words of Dr. G. Hayward, "the proposition to mitigate the severity of the law against those engaged in dissection, was driven almost by acclamation from the legislature."

Subsequent legislation has considerably modified the act of February 28, 1831, as may be seen on consulting the Acts of April 1, 1834, March 26, 1845, May 10, 1855, and March 28, 1857.   By the Act of 1845, chapter 242, former Acts are simplified, amended and improved.   Section 1 provides that the overseers of the poor of any town, and the mayor and aldermen of any city, in the commonwealth, " shall, upon request, give permission to any regular physician, duly qualified according to law, to take the dead bodies of such persons as are required to be buried at the public expense within their respective towns or cities ; " and also makes it " the duty of all persons having charge of any poorhouse, work-house, or house of industry, in which any person required to be buried at the public expense shall die, immediately to give notice thereof to the overseers of the poor of the town, or the mayor and aldermen of the city, ... and the dead body of such person shall not, except in cases of necessity, be buried, nor shall the same be dissected or mutilated until such notice shall have been given and the permission therefor granted."   According to section 2, " no such body shall in any case be surrendered if the deceased person, during his last sickness, of his own accord, requested to be buried."   Excepting the repeal of sections 10 and 11 of the Revised Statutes of 1835, the Act of 1845 contains no other noteworthy new provision.

Chapter 323, Laws of Massachusetts, 1855, section 1, confers the powers and duties of overseers of the poor, as defined in chapter 242, Laws of 1845, upon " overseers and superintendents of State almshouses."   Section 2 contains provisions new to the statute book.   It reads : " Whoever buys, sells, or has in his possession for the purpose of buying, or selling, or trafficking in, the dead body of any human being shall be punished by fine of not less than fifty, nor exceeding five hundred dollars, or by imprisonment in the jail not less than three months, nor exceeding three years."   The duty of giving immediate notice to the proper authorities of the death of friendless persons in the institutions under their control, devolved by the Act of 1845 upon the directors of houses of industry, etc., etc., is also, by the Act of March 28, 1857, laid upon the board of directors of public institutions of Boston.

So far as the writer has been able to learn, the Massachusetts legislature has enacted nothing of interest concerning anatomical science since 1857.

We have already noticed the provisions of the Act of 1784, concerning the dissection of dead duellists. The Act of 1784 was repealed March 15, 1805, when the following was enacted : . . . . . "Justices of said court, before whom the conviction shall be, shall in cases of murder committed in a duel, and in other cases, may, at their discretion, further sentence and order the body of such convict to be dissected and anatomized."

In chapter 125, section 2, page 716, Revised Statutes 1835, we find no mention of "murder committed in a duel;" but we do find that "in every case of a conviction of the crime of murder, the court may, in their discretion, order the convict to be dissected, and the sheriff shall deliver the dead body of such convict to a professor of anatomy and surgery in some college or public seminary, if requested; otherwise it shall be delivered to any surgeon who may be attending to receive it, and who will engage for the dissection thereof." The last revision of the Massachusetts statutes contains the above provision for the dissection of a dead murderer's body, practically unchanged, excepting this saving clause: "unless his friends desire it for interment."

The Massachusetts Anatomy Act of 1831, was productive of results in two directions; it lightened the burdens of the teachers of anatomy in that State, and it led to the enactment of similar laws in other States. Connecticut passed a liberal Act, modelled on that of Massachusetts, June 5, 1833, but repealed the same June 5, 1834. New Hampshire legalized anatomy in 1834, but rescinded its action in 1842. Michigan passed "an Act to facilitate the study of anatomy," March 9, 1844, but repealed it April 7, 1851. New York is entitled to the place of honor next to Massachusetts, on the list of States which have consistently endeavored to promote anatomical science. The New York law of April 1, 1854, has never been repealed; on the contrary, it has been improved, notably by the amendatory Act of June 3, 1879.

Referring to the Massachusetts law of 1831, as amended in 1845, Dr. John C. Warren, says: "The Superintendent of the House of Industry opposed great difficulties to the execution of this law; but he dying in 1847, an ample supply was obtained for the medical school afterwards, particularly in consequence of the influx of Irish paupers, and the great mortality among them." Concerning the working of the same law; Dr. George Hayward, writing in 1855: "The supply has not been, perhaps, as great as

could be wished; but, with the increase of population and pauperism, this objection will pass away." We doubt, if in the judgment of the anatomists of the Harvard Medical School, "this objection" has "passed away." We incline to the belief that "with the increase of population and pauperism," there has been, at least, an equal increase of demagogues, and that no class of men in Massachusetts have a more realizing sense than have its anatomists of the relation existing between eternal vigilance and the price of liberty.

The city government of Boston, November 3, 1869, ordered "that permits be issued by the city clerk, until otherwise ordered, to the surgeons of the Harvard Medical School to take the dead bodies of such persons dying at Deer Island, or the House of Correction, the County Jail, or City Hospital, as may be required to be buried at the public expense." The statutory restrictions concerning the delivery of unclaimed bodies are embodied in the remainder of the ordinance. The anatomists of Baltimore, Washington, and New Orleans, might fairly consider this Boston ordinance a liberal one, for they are still obliged to dissect without legal warrant, or not at all. On the other hand, in Germany or France, where for years the dissecting rooms have been furnished with the unclaimed dead by the police, this ordinance would, unquestionably, be considered imperfect and illiberal.

It is unfortunate that American anatomists are forced to dance attendance upon public functionaries for "permits;" as they are thereby put in the false position of seeking as a personal favor what ought to be furnished them for the furtherance of the public welfare. Possibly, the time is not yet ripe for the Massachusetts anatomists to demand that the unclaimed dead of Springfield, Fall River, Worcester, Lowell, in short, the entire State, as well as of Boston, should be delivered to them at their dissecting rooms; but such a consummation is none the less devoutly to be wished. Massachusetts led off in legalizing the dissection of bodies required to be buried at the public expense. Would that she might inaugurate an administrative reform which should prevent the present wasteful decomposition of valuable material at the bottom of graves, and preclude the necessity which requires one who is bent on thoroughly learning practical anatomy in all its branches, to seek the anatomical institutes of Europe.

The legal status of anatomy in America, at the beginning of the century, is well illustrated by the Connecticut Acts of 1810.

At the May session of that year, it was made punishable by a fine of at least one hundred dollars and imprisonment in the county jail for at least three months, for any one secretly to disinter the body of any deceased person for the purpose of dissection, or in any way to aid in so doing, or knowingly "to assist in any surgical or anatomical experiments therewith or dissections thereof." At the October session it was enacted that there should be a "medical institution of Yale College," one of whose four professors should teach anatomy, surgery, and midwifery; and that, as speedily as the college funds would allow, a collection of anatomical preparations should be provided.

The Massachusetts Act of 1784 only authorized dissection of dead duellists as a mark of infamy; therefore, the New York Act of 1789 must be considered as the first American anatomy law. This Act was passed the year after the famous "Doctors' Mob" in New York city, and is entitled, "An Act to prevent the Odious Practice of Digging up and removing, for the purpose of Dissection, Dead Bodies interred in Cemeteries or Burial Places." It comprises two sections. Section I. provides that any person convicted of removing any dead body from its place of sepulture, for the purpose of dissection or with intent to dissect, or of dissecting or assisting to dissect, such body, "shall be adjudged to stand in the pillory or to suffer other corporal punishment, not extending to life or limb, and shall also pay such fine and suffer such imprisonment as the court shall in their discretion think proper to direct." In Section II. it is further enacted, "In order that science may not in this respect be injured by preventing the dissection of proper subjects, that when any offender shall be convicted of murder, arson, or burglary, for which he shall be sentenced to suffer death, the court may, at their discretion, add to the judgment that the body of such offender shall be delivered to a surgeon for dissection." Massachusetts made the first considerable improvement on this New York Act when in 1831, it passed a statute authorizing, under certain restrictions, the delivery to the anatomists of the unclaimed bodies "of deceased persons required to be buried at the public expense."

Enactments similar to the New York Act of 1789, Section I., have since been passed by the following States: Alabama, Arkansas, California, Connecticut, Georgia, Illinois, Indiana, Iowa, Kansas, Kentucky, Maine, Massachusetts, Michigan,

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Minnesota, Mississippi, Missouri, Nebraska, New Hampshire, Ohio, Oregon, Pennsylvania, Rhode Island, Tennessee, Texas, Vermont, Virginia, West Virginia, and Wisconsin. Of the above-mentioned States, Kentucky, Oregon, Rhode Island, Texas and West Virginia have no anatomy Acts; while Rhode Island, Texas, and West Virginia have no medical schools. The laws of nine States, namely, Colorado, Delaware, Florida, Louisiana, Maryland, Nevada, New Jersey, North Carolina, and South Carolina, are, so far as the writer has been able to learn, silent regarding grave-robbery. While the Territories of Dakota, Utah, Washington, and Wyoming have laws for the protection of sepulchres, the District of Columbia has no such law, although one was inserted into the proposed code of 1857, which failed of adoption.

The second section of the New York Act of 1789 has developed into the Acts of twenty-four States. The following named States have legalized dissection: Alabama, Arkansas, California, Colorado, Connecticut, Georgia, Illinois, Indiana, Iowa, Kansas, Maine, Massachusetts, Michigan, Minnesota, Missouri, Nebraska, New Hampshire, New Jersey, New York, Ohio, Pennsylvania, Tennessee, Vermont, and Wisconsin.

The dissection of executed criminals, as such, is still lawful within the special jurisdiction of the United States Government and in the following States: Alabama, Arkansas, Colorado, Connecticut, Georgia, Illinois, Indiana, Kansas, Massachusetts, Missouri, Nebraska, and New Jersey. Nebraska, like the United States and New Jersey, makes no provision other than this for its anatomists. Unlike them, however, it has a penal statute regarding grave robbery. Alabama, Georgia, Missouri, and Tennessee allow the dissection not only of executed criminals, but also of "other persons with the consent of their friends."

Kentucky, Mississippi, Oregon, Rhode Island, Texas, Virginia and West Virginia are without laws of any kind regarding dissection, though they all forbid violation of sepulture. The most backward of the United States are those which have no statute touching either dissection or grave robbery. In this class we find Delaware, Florida, Louisiana, Maryland, Nevada, North Carolina, South Carolina and the District of Columbia. The Territories of Arizona, Idaho, Montana, and New Mexico are similarly indifferent to the science of anatomy and the sanctity of burial-places. Certain of these States, like Maryland, Louisiana and South Caro-

lina, contain medical schools. In order to punish body-snatching Maryland is to-day obliged to fall back on the common law of England, although the common law penalty was superseded nearly fifty years since, by the passage of the Warburton anatomy act. All things considered, the attitude of the Italian cities of the fourteenth century and that of the empire of Japan of to-day must be characterized as more liberal and enlightened regarding the alphabet of medicine than that of the United States and of very many of the individual States.

The Acts of the following States may be termed fairly liberal: Arkansas, California, Connecticut, Illinois, Indiana, Iowa, Kansas, Massachusetts, Michigan, Minnesota, New Hampshire, New York, Ohio, Pennsylvania, and Wisconsin.

The Acts of Alabama, Colorado, Georgia, Maine, Missouri, Nebraska, New Jersey, Tennessee, and Vermont are illiberal.

In 1869 Maine enacted "that when any person convicted of crime dies or is executed in the State prison or any jail, the warden or keepers shall, on request, deliver his body to instructors in medical schools established by law." In February, 1876, capital punishment was abolished; so that at present in Maine it is legal to dissect only the body of a person who "requests during his life that his body may be delivered to a regular physician or surgeon for the advancement of anatomical science, after his death, unless some kindred or friend within three days" asks to have it buried; or the body of a convict who has not at any time requested to be buried, and whose friends and kindred fail for three days after his death to ask for his burial.

The statute of Tennessee, unless it has been repealed since 1871, is quite as liberal as that of Maine. It provides that no penalty shall "apply to regular physicians to whom the bodies of criminals are delivered pursuant to law, or to dissection of slaves by consent of their masters, or of other persons by consent of their relatives."

The New York Act of June 3, 1879, may be instanced as a type of the liberal class of American Acts. It reads: "It shall be lawful in cities whose population exceeds 30,000 inhabitants, and in counties containing said cities, to deliver to the professors and teachers in medical colleges and schools in this State, and for said professors and teachers to receive, the remains or body of any deceased person for the purposes of medical and surgical study: provided that said remains shall not have been regularly interred,

and shall not have been desired for interment by any relative or friend of said deceased person within twenty-four hours after death; provided, also, that the remains of no person who may be known to have relatives or friends shall be so delivered or received without the consent of said relatives or friends; and provided that the remains of no one detained for debt, or as a witness, or on suspicion of crime, or of any traveller, nor of any person who shall have expressed a desire in his or her last sickness that his or her body may be interred, shall be delivered or received as aforesaid, but shall be buried in the usual manner; and provided, also, that in case the remains of any person so delivered or received shall be subsequently claimed by any surviving relative or friend they shall be given up to said relative or friend for interment. And it shall be the duty of the said professors and teachers decently to bury in some public cemetery the remains of all bodies after they shall have answered the purposes of study aforesaid; and for any neglect or violation of this provision of this Act the party so neglecting shall forfeit and pay a penalty of not less than $25 nor more than $50, to be sued for by the health officers of said cities, or of other places, for the benefit of their department." An earlier law of New York forbids all traffic in subjects, or any use of them, except for anatomical purposes, under penalty of imprisonment in jail for not more than a year.

To summarize the legislation from 1789 to 1881, we may say that twenty-four States allow dissection; fifteen States have liberal anatomy Acts, while nine have illiberal ones; the laws of fourteen States are silent regarding anatomy, excepting their laws on malpractice; twenty-eight States forbid the desecration of graves, while the laws of ten States are silent regarding it; the laws of the six States are silent touching both dissection and disinterment; Dakota alone of the eight Territories allows dissection; four Territories forbid exhumation, and four have no enactment regarding it; twelve States and one Territory require the burial of *cadavera dissecta.*

The District of Columbia occupies a unique position among the capitals of civilized States in that the studies of its anatomists and the graves of its dead are alike unprotected by statutory enactments. The United States government sends Washington resurrectionists to jail when it can; but it has recently utilized in the examinations before the Navy Board, in the city of Washington,

as many as twelve subjects, which could be procured by stealth only.

The most elaborate, the most liberal, and also the most stringent of the American anatomy Acts have been passed within the last five years. Those of Indiana, Ohio, and New York, were passed in 1879; and the amended Act of Iowa, March 26, 1880. So far as I can learn the amended Michigan Act, approved March 2, 1881, is the latest American Anatomy Act.

The Michigan Act of 1844, which, as we have noticed, was repealed in 1851, required the officers of the State prison to surrender the bodies of all unfriended convicts dying in their prison to any agent of the medical society of the State who should present an order for the same signed by the president of the society. Similarly, the unclaimed bodies of convicts dying in a county jail, under sentence of six months imprisonment or more, were deliverable to the agents of the medical society of the county in which the jail was situated. In 1867 a new Act was passed, which has since been thrice amended,—once in 1871, again in 1875, April 27th, and again by the Act of March 2, 1881. The last mentioned Act contains provisions which render it the most advanced and liberal of all American Anatomy Acts; I therefore give it in full.

"SEC. 1. *The People of the State of Michigan enact,* That sections 1 and 2 of act number 138 of the session laws of 1875, approved April 27, 1875, being sections 2110 and 2111 of chapter 65 of the compiled laws of 1871, as amended, be amended so as to read as follows:

"(2110). SEC. 1. Any member of either of the following boards, and any of the following named officers or persons, to wit: The board of health of any city, village, or township, the common council of any city, the board of trustees of any village, the mayor of any city, president of any village, any board, or officer having the direction, management, charge, or control, in whole or in part, of any prison, house of correction, work-house, jail, or lock-up, county superintendents of the poor, keepers of poor-houses and almshouses, any physician, or other person in charge of any poor-house or almshouse, sheriff, coroners, the board of State commissioners, the board of trustees, board of control, and all officers, physicians, and persons in charge, in whole or in part, of any institution for the deaf and dumb, blind, and insane, or other

charitable institution founded or supported, in whole or in part,
at public expense, having in his or their possession or control the
dead body of any person not claimed by any relative, or legal
representative, as hereinafter provided, and which may be required
to be buried at public expense, or the expense of any one of such
public or charitable institutions, shall deliver such dead body or
bodies, within thirty-six hours after death, or after he or they
shall become possessed thereof, to the express or railway company
at the nearest railway station, placed in a plain coffin and enclosed
in a strong box, securely fastened, and plainly directed to the
" Demonstrator of anatomy, of the University of Michigan, Ann
Arbor, Mich.," excepting only the dead bodies of such persons as
shall have died from some infectious disease.   And such boards,
common councils, officers, or other persons making such shipment
shall take the usual shipping receipt for such package, and shall
notify the consignee of such shipment by letter, mailed on the day
the package is so delivered as aforesaid ; and shall also inclose in
such letter a statement giving, as nearly as can be ascertained, the
name, age, residence, and cause of death of such deceased person ;
and the name and postoffice address of the known relatives of such
deceased person, whose body has been shipped as aforesaid ; and
also a statement of the costs and expenses which have been incurred
in the procuring of the coffin, box, preparation of body for ship-
ment, and shipping the same.   And, upon the receipt of such con-
signment, the said demonstrator of anatomy of the University of
Michigan shall immediately forward to such officers, board, coun-
cil, or institution, or persons making such shipment, or incurring
such expenses, the amount thereof, not exceeding in any case the
sum of fifteen dollars : *Provided,* Such dead body shall not be so
shipped or delivered as aforesaid, if it shall be requested in good
faith for interment by any relative before the same shall be shipped
as aforesaid, and in case the dead body of any person, so delivered
or shipped as aforesaid, be subsequently claimed or demanded of
said demonstrator of anatomy, or of any other person or institu-
tion, into whose possession or under whose control it may have
been placed, by virtue of the provisions of this law, by any rela-
tive or legal representative of such deceased person, for private
interment, it shall be given up to such claimant even after the
same shall have been interred, as hereinafter provided.   Such
bodies shall be used only for the purposes hereinafter mentioned,

and shall then, in all cases, be interred in some suitable place, kept for that purpose, and a correct record shall be kept of every such body, and all matters by which such body may be identified coming to the knowledge of the person or officer at any time in charge of such bodies, shall be faithfully recorded at length in a book to be kept for such purposes, to the end that the same may be at any time traced and recovered by the friends and relatives of such deceased person : *And provided further,* That the institution, board, council, officer, or person aforesaid in charge of any such body as aforesaid shall, immediately after the death of such person, notify, if possible, by telegraph, or otherwise by letter, one or more of the nearest known relatives of such deceased person of the death of such person ; and in no case shall the body of any such deceased person be delivered or shipped as aforesaid until after the expiration of twenty-four hours from death ; and every individual officer or party violating any of the provisions of this section shall be deemed guilty of a misdemeanor.

"(2111). SEC. 2. The bodies so delivered, or shipped as aforesaid, shall be used for the advancement of anatomical science in this State and in the following institutions of learning only, viz : The University of Michigan, Detroit Medical College, and Michigan College of Medicine. And said bodies shall be distributed to and among the same equitably, the number assigned to each by said demonstrator of anatomy, shall be proportional to that of its students in actual attendance. And each of said institutions shall pay quarterly to said demonstrator its ratable proportion of the expenses borne and incurred under this act : *Provided, however,* That said demonstrator of anatomy, upon the receipt of every body, under and by virtue of the provisions of this act, shall cause the same to be embalmed or put in a state of preservation, and shall not permit the same to be delivered to either of said institutions for the purpose of dissection, until the same shall have been in his possession at least ten days. And it shall be the duty of said demonstrator of anatomy, upon the receipt of every body, to immediately notify the relatives of such deceased person, if known, of the receipt of such body, either by mail or telegraph, as he may deem best. And that said body will be preserved intact, for the space of ten days, in which time such relative will be entitled to said body for the purpose of private interment, upon payment of the expenses already incurred. And if the relatives

or legal representative of such deceased person shall request said body for the purpose of interment, and shall pay said expenses, said demonstrator shall deliver to such relative or legal representative the said body, together with the said coffin and box enclosing the same. But in case said body shall not be requested by such relatives until after the same shall have been applied to the purposes intended, the remains thereof, together with the coffin and box aforesaid, shall be delivered without charge: *Provided,* That the University of Michigan, Detroit Medical College and Michigan College of Medicine aforesaid, and each and every other medical institution shall not receive into their possession any bodies procured in this State other than those provided for by the provisions of this act, and every individual or party violating this provision shall be deemed guilty of a misdemeanor."

Indiana had not legalized dissection when, in the spring of 1878, the body of the Hon. J. Scott Harrison, a son of the late William Henry Harrison, President of the United States, having been stolen from its grave near to the Ohio line, was found by the son of the deceased, the day after his burial, in a Cincinnati dissecting-room, whither he had gone in search of another body. The only penalty for grave-robbery under the Indiana statutes was a fine not exceeding one thousand dollars, provided by the Act of June 14, 1852. This case of resurrecting led to the improvement in 1879 of the laws of both Indiana and Ohio. Possibly the stringent amendment to the Iowa law, passed March 26, 1880, might be traced to the outrage of the Harrison tomb.

Chapter LXV. of the laws of the fifty-first session of the General Assembly of Indiana is "an Act in relation to the use of human bodies for the purpose of dissection; to require a record thereof to be kept, and to punish the unlawful possession or dissection of such bodies and the violation of graves."

Section 1 requires that all institutions or persons engaged in dissection shall keep a record book containing full particulars regarding every corpse received for dissection.

Section 2 makes it punishable by a fine of "not less than one hundred nor more than five hundred dollars, to which may be added imprisonment in the county jail for any period not less than one month nor more than one year," if the person having the custody of the record required by section 1 fail or refuse to produce it. Section 3 declares it a felony, punishable by impris-

onment for not less than one nor more than five years, for any person to "receive, or have in possession, or dissect, or permit to be dissected, ... any such body of which the record required by section 1 shall not have been made." Making a false entry in the record is made a felony by section 4, punishable by not less than one nor more than three years' imprisonment in the State prison.

Imprisonment in the State prison for not less than two nor more than five years is the penalty provided by section 5 for the felon "who shall dissect, or have in his possession for the purpose of dissection, any human body, or any part thereof, other than such as are or may be given by law for such uses." Section 6 makes those who "have the supervision of the dissecting-room and of the instruction given therein" responsible "for bodies received or found therein." Section 7 relates to illegal exhumation which is made a felony, punishable by imprisonment in the State prison "for not less than three nor more than ten years." According to section 9, one who knowingly aids in concealing an unlawfully procured body is liable, as a felon, to imprisonment in the State prison for from one to three years. Section 10 declares that "any person who shall buy or receive, by gift or otherwise, any dead human body, or any part thereof, knowing the same to have been disinterred . . . . . in violation of this act, shall be deemed an accessory to such offense, and, on conviction thereof, be punished in like manner as is prescribed in the preceding section."

Chapter LXVI of the Session Laws of Indiana for 1879, is an Act "to promote the science of medicine and surgery by providing methods whereby human subjects for anatomical and scientific dissection and experiment may be lawfully obtained, and prescribing penalties for violation thereof." The Act is a liberal one. The penalties provided for its violation are severe. Its fifth section is unusual in its provisions.

"SECT. 5. In case of any vagrant found dead, or in case of any person killed while committing a felony, or if any prisoner is convicted of felony and justifiably killed in attempting to escape from prison or from officers of the law having him or her in lawful custody, upon the body of which person an inquest may lawfully be held, and shall be held by the coroner or other officer thereto lawfully authorized, it shall be the duty of such inquest to inquire

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as to the existence and residence of any next of kin of such deceased person ; and if it shall be the verdict of such inquest that the person so found dead or killed had no next of kin, the coroner or other officer holding such inquest may at his discretion, and with the approval of the sheriff of the county wherein such inquest is held, upon the request in writing of the faculty or other authorities of any duly incorporated and organized medical college or medical association within this State, in operation nearest to the place of such inquest, deliver such dead body to such college for the scientific purposes thereof, taking a proper descriptive receipt therefor, which shall be filed with the clerk of the county."

Ohio, as early as 1831, enacted penalties for grave robbery, but did not pass any "Act to encourage the study of anatomy," till March 25, 1870, when an inadequate law with the above title was passed.   House bill No. 216, Ohio legistature, 1878, embodied an attempt to repeal the Act of 1870, in the following remarkable terms :—

"*Whereas*, by the laws of this State the bodies of criminals, executed for heinous offences, unless said criminals are poor and friendless, are entitled to decent burial ; and *whereas*, poverty is no crime, and the poor, honest, friendless man, in life and in death, should before the law be the equal, at least, of the depraved criminal ; and *whereas*, by the laws of this State the bodies of deceased and unclaimed poor are authorized to be given over to certain colleges and schools for dissection ; therefore,—

"SECT. 1. *Be it enacted*, etc., That an Act entitled 'An Act to encourage the study of anatomy,' passed March 25, 1870, be and the same is hereby repealed.

"SECT. 2. This Act shall take effect and be in force from and after its passage."

The person who introduced this bill, meeting with unexpected opposition, finally withdrew it, saying that he had "only introduced it for fun."   The Harrison horror satisfied the Ohio legislators that anatomy could not be regulated by jocose legislation ; and an earnest attempt was made to protect alike the anatomists and the dead, as may be seen on consulting section 3763 of the Revised Statutes of Ohio, 1880.

From 1851 till 1880 it was provided, in the chapter of the code of Iowa which relates to offenses against chastity and decency, that every offender who should illegally disinter, or assist in disinter-

ring or concealing any human body, should "be punished by imprisonment in the county jail not exceeding one year, or by fine not exceeding $1000, or by both fine and imprisonment." By Act of March 26, 1880, embodied in section 4019½ of Revised Statutes of Iowa, every such offender is now liable to imprisonment "in the penitentiary not more than two years, or by fine not exceeding $2500, or by both fine and imprisonment." By the Act of April 22, 1872, it is allowed in Iowa, under the customary restrictions, for any coroner or undertaker in any county or city in which the population exceeds one thousand inhabitants to deliver to any medical college or school, or any physician in the State, for the purpose of medical or surgical study, the body of any deceased person, except where such body had been interred or dressed for interment.

I have endeavored to ascertain some facts as to the amount and cost of the dissection done in our American schools of medicine. I can find no statistics on the question. The following statement is based on the figures of the forthcoming report for 1879 of General John Eaton, United States Commissioner of Education, and on such data as have been kindly furnished me by several prominent teachers of anatomy. The total number of medical students of "all sorts" in the United States, in 1879, was 13,321, showing an increase of 1,484 over 1878, and of 7,378 over 1870. Of these 9,603 were in attendance upon 988 instructors in 68 so-called regular schools, in 26 States and the District of Columbia. The increase of regular students in 1879 over 1878 was 1,317. In 12 States with liberal anatomy laws there were 34 schools, with 599 instructors and 5,294 students.

Indiana and Ohio joined the column of liberal States in 1879, with a total number of 1,219 students; whereas in 1878 the total number in those States was 945. In 6 States with illiberal laws there were 18 schools with 228 instructors and 1,672 students; and in 8 States and the District of Columbia there were 1,652 students in 15 schools, with 122 instructors, unprotected by law in the study of practical anatomy. Kentucky, with 4 schools and 603 students, had no anatomy law. The District of Columbia had 158 students in 3 schools; also 1 President of the United States and 1 Congress, ditto, but no anatomy law. Maryland with 2 schools and 468 students; Louisiana with 1 school and 193 students; South Carolina with 1 School and 71 students;

and North Carolina with 1 school and 7 students had no anatomy Act and no statute forbidding disinterment of the dead. The city of Baltimore buried 577 unclaimed dead bodies in 1880, while her anatomists were obliged to use stolen subjects or none.

During the winter of 1879-80, in 11 medical schools in 6 States and the District of Columbia, there were 1,944 students in attendance, of whom 1,255 dissected, and 609 dissected more than one "part." On the average the dissection of two parts is required for a degree. The average cost of a part was $3.00, the extremes being $9.00 and nothing. The demonstrator's ticket is not reckoned in the cost per part. The average cost of subjects to the schools was $18.72; the extremes of price being $3.00 and $50.00. Usually 5 students dissect on a single subject, but in one school 8 and in another 10 students work on the same subject, alternately reading and dissecting. Of 445 subjects used, not more than 39 were used by students in making surgical operations on the cadaver. Three only of the eleven schools claim to prescribe such a course of operations; but judging from the number of students who took it, it is a medical rather than a legal prescription. Of the 1,255 students who dissected, 465 using 133 subjects were unprotected by law in so doing. On the basis indicated above, it is computed that between 3,400 and 3,500 subjects should have been used by the students in the regular medical schools of the United States. The official returns show that in France in 1876, 3,463 subjects were delivered in accordance with law, at the anatomical theatres of schools having an aggregate of 5,624 students.

We have traced, thus far, the course of practical anatomy in America from the time of Giles Firmin till the close of the last century; and have considered in a more detailed way the development of what may be characterized as the most typical of the American Anatomy Acts, namely, the Massachusetts law. The same obstacles of prejudice and apathy which beset the anatomists of our younger States, have been operative in every land where anatomy has gained a foothold, since the days of Ptolemy. It would be interesting to attempt to analyze the popular prejudice against human dissection, which prejudice is a strange compound of pagan superstition, Christian materialism, and an innate aversion to the morals, aims, and manners of the average American medical student. Such an attempt would take us too far afield. It is note-worthy, however, that anatomy has flourished chiefly under

the rule of princes and prelates. Anatomists have usually found republics, to say the least, ungrateful. We ought not to be surprised, therefore, when we consider American Anatomy Acts as a class, to find certain of our States no more enlightened in this regard than was France when Vesalius had to contend by night with vultures and prowling dogs for the carcase of the murderer or the suicide. The utmost help that several of our States give to anatomists is the occasional gift of the body of an executed malefactor; while others of them have not attained even to that mediæval stage of generosity.

The guild spirit which led to the incorporation of the Edinburgh Surgeons as a "Company," in 1505, and the incorporation of the "Mystery and Commonalty of Barbers and Surgeons of London," in 1540, may be said to characterize the majority of our American medical colleges which are, as has been well said by President Eliot of Harvard University, managed as commercial ventures. This trading monopolizing spirit is more marked in British than in Continental schools of medicine. The radical difference between European and American medical education results from the woeful lack, on this side of the Atlantic, of the well-considered, consistent, and responsible State supervision exercised over the teachers, students, and practitioners of medicine in most European countries. In no department of medical education is this difference more strongly marked than in that of anatomy. It is equally clear whether we consider the training and attainments of the teachers, the amount of practical knowledge required of the students, or the laws regulating the supply of material in this department.

It is no less certain that the German and French schools of anatomy outrank the British, than that the latter outrank the American. While one might, from sources to be found in the libraries of Washington, Boston, and Baltimore, trace the development of the French laws concerning the cadaver, I find it impossible to make any detailed statement, based on authentic documents, regarding the laws which regulate the organization and maintenance of the German institutes of anatomy. It may be stated, however, that an Act which should embody the best features of the best American Anatomy Acts, while it would compare favorably with the British laws, would fall far short of the French, in point of comprehensiveness and liberality; and it is safe to say

that no medical school in the United States combines the rigid requirements of Vienna and Prague, of seventy years ago, with anything like the wealth of opportunity offered to-day at Paris and Bonn. One who should desire to become a thoroughly éxpert anatomist through the dissection of the dead rather than by mangling the living, would be justified in going from America to Germany or France simply on grounds of economy. The depopulation of American medical colleges, owing to such a cause, need, however, not be feared, so long as the present public and professional indifference to ignorance of the fundamental facts of medical science obtains.

ALTERNATION OF PERIODS OF REST WITH PERIODS OF ACTIVITY IN THE SEGMENTING EGGS OF VERTEBRATES. By W. K. BROOKS, Ph. D., *Associate in Biology.* With Plate VIII.

In the first volume of this Journal I have called attention to the fact that the well-known contraction of the molluscan egg after each division is the external indication, at least in the Fresh-water Pulmonates and the Oyster, of an alternation of periods of rest with periods of activity.

I have suggested, (Vol. I, No. 2, page 78), that this alternation may be due to the need for an accumulation of energy, by the assimilation of the food contained in the egg, in order to overcome the physical resistance of the protoplasm.

According to this view the separation of the periods of activity by intervening periods of rest is the essential feature, and the contraction after each division a secondary phenomenon; and it is therefore interesting to find, in eggs where the blastoderm is small and the food yolk large and inelastic, that while there is no contraction after each division there is, during the early stages at least, a well marked period of rest after each period of activity.

During the summer of 1880 I obtained, at the marine laboratory of the Johns Hopkins University, a number of large fish-eggs, which are probably those of Batrachus tau (Linn.) While many of the eggs appeared to be perfectly healthy, and while I found them in various stages of segmentation, I at first failed to observe any change whatever in a single egg, even after several hours observation.

As it seemed possible that this might be due to the rapidity of the change when it did take place, I determined to keep a single egg under constant observation until I saw it undergo segmentation, or satisfied myself that it was dead, and the result was quite interesting, since it showed that the periods of change, which are rather short, are separated from each other by extremely long periods of rest.

The blastoderm of the egg which was selected is shown in Figure 1, Plate VIII, as seen from above, magnified eighty diameters. It is divided into eight spherules, which are sym-

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metrically placed on the sides of a longitudinal axis. At one end
of this axis there are two large spherules 1, and, following these,
a second, somewhat larger pair 2; then a very small pair 3, and
at the opposite end of the axis a fourth pair 4, nearly, but not
quite, as large as the first pair. This egg was so perfectly sym-
metrical, and its spherules so well defined, that I felt sure that it
was alive, and therefore determined to keep constant watch of it
until some change took place. I do not know how long it had
been in this condition before I placed it under the microscope, but,
for two hours after, it exhibited no visible change whatever. At
the end of this time nuclei became visible in the cells 4 and 3 and
soon divided, and at the end of five minutes each of the spherules 3
had divided into two, as shown in Figure 2; each of the spherules 4
had a double nucleus, one of the cells 2 a double nucleus, and the
other a single one.

In five minutes more, Figure 3, all the spherules were in some
stage of division, but this was more advanced on one side of the
axis than on the other. In five minutes more, Figure 4, all the
spherules except 2 and 4 on one side were perfectly divided. In
ten minutes more, Figure 5, the division was completed; the
blastoderm was divided into sixteen spherules, and these were
symmetrically arranged in pairs, on the two sides of a long axis,
which was identical with that of Figure 1.

The perfect bilateral symmetry of this stage formed such a
marked contrast to all the stages between it and Figure 1, that I
felt confident that it marked the end of a period of segmenting
activity, and that a period of rest would now follow.

The result fully justified this supposition, for, although I
watched it for more than three hours, no more change was visi-
ble, and when I retired at night it was as shown in the figure.

It was not dead, for the next morning the blastoderm was found
to be divided up into a great number of small cells, as shown in
Figure 6, which is a little more magnified than the other figures.

During the summer I observed the same phenomenon in the
segmenting egg of an Arthropod, and it was observed by Mr.
Wilson in Annelid eggs. Dr. Clarke has also observed it in
Amblystoma, and I think we may conclude that it is charac-
teristic of segmentation in general; that wherever circumstances
admit of a careful time-record, the active changes will be found to
be separated from each other by periods during which there is no
visible external change.

A NEW METHOD OF STUDYING THE MAMMALIAN HEART.
By H. NEWELL MARTIN, M. A., D. Sc. M. D. With Plate IX.

In the course of some experiments made by me in conjunction with Dr. W. T. Sedgwick, on blood pressure in the coronary arteries of the heart, the fact was impressed upon me that the mammalian heart is no such fragile organ as one is usually inclined to assume, but possesses a very considerable power of bearing manipulation. On the other hand, I knew of various unsuccessful attempts to isolate the mammalian heart and study its physiology apart from the influence of extrinsic nerve centres, in a manner more or less similar to the methods so frequently used for physiological investigations on the heart of a cold-blooded animal; the mammalian heart, however, always died before any observations could be made on it. Thinking over the apparent contradiction, it occurred to me that the essential difference probably lay in the coronary circulation; in the frog, as is well known, there are no coronary arteries or veins, the thin auricles and spongy ventricle being nourished by the blood flowing through the cardiac chambers, but in the mammal the thick-walled heart has a special circulatory system of its own and needs a steady flow through its vessels, and cannot be nourished (as appears to have been forgotten) by merely keeping up a stream through auricles and ventricles. The greater respiratory needs of the heart of the warm-blooded animal also needed consideration; the lungs ought either to be left connected with it, or replaced by some other efficient aërating apparatus; if entirely separated from the central nervous system there seemed no need to replace the natural lung by an artificial one, and, though I hope ultimately to do this, my work hitherto has been confined to the study of heart and lungs living together, when all the rest of the body of the animal was dead. Under such circumstances, with uniform artificial respiration, the lungs may be regarded as purely physical organs adapted for gaseous diffusion; and probably better for this purpose than any substitute which could be constructed.

My first experiments were made with cats. The animal was narcotised with morphia, tracheotomised, and a cannula put in the

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left carotid. Then the thorax was opened, (artificial respiration being started), the innominate artery tied beyond the origin of the left carotid but proximal to the point where the right subclavian and right carotid separate; the left subclavian was ligatured near its origin; and the aortic arch tied immediately beyond the organ of the left subclavian. Finally, the superior and inferior cavæ and the root of one lung were tied; the cannula in the left carotid was connected with the manometer of the kymographion, and tracings taken in the usual manner. Under these circumstances the course of the blood was—left auricle, left ventricle, aortic arch and the ligatured arterial stumps connected with it, the coronary vessels, the right auricle, the right ventricle, the pulmonary circulation through one lung, and back to the left auricle. All circulation was cut off from every organ in the body except heart and lungs; the brain and spinal cord soon died, the muscles became rigid, and kidneys and liver had no longer any physiological connection, either through the nervous system or the blood, with the heart; which, though still in the body, was physiologically isolated from everything but the lungs; yet as my preliminary experiments shewed (Johns Hopkins University Circular, No. 10, p. 127, April, 1881,) the heart went on beating with considerable force and regularity for more than an hour.

The method, however, still left much to be desired; I wanted the heart alive much longer; a means of keeping it at a uniform temperature; a method of renewing the blood which, either because clogged with waste products usually removed by the kidneys or other organs, or because certain nutritive materials in it were used up, ceased to be efficient in keeping the heart alive after a certain time; and opportunity to run blood, to which various substances had been added, through the heart from time to time in order to study their action upon it.

After several attempts the apparatus represented in Plate IX was devised, and has been found to answer admirably; with it I have kept a heart, isolated physiologically from everything but the lungs, beating with beautiful regularity for more than five hours, and have no doubt I could keep it considerably longer were that necessary.

In the plate the heart is represented very diagrammatically and of hugely disproportionate size; the pulmonary vessels also are entirely omitted, as they are not interfered with in the experiment.

At first I thought the immense disproportion in capacity between the complete pulmonary system of vessels and the systemic circulation reduced to only its coronary portion would injure the working of the heart, and I tied up, as above stated, the root of one lung and sometimes one or two lobes of the other; but I have since found that this is quite unnecessary; the left auricle takes only what it wants, no matter how much blood is accumulated in the lungs, and the circulation is thus confined to the quantity of blood which under a given aortic pressure is sent through the coronary system in a given time.

The course of an experiment is as follows: Tracheotomy having been performed, each pneumogastric nerve is divided in the neck; this is, I find, of importance as saving the heart from the effects of powerful dyspnœic inhibition when subsequently all the cerebral circulation is cut off. A cannula, *p*, is then placed in the left carotid, *o*; and another, *s*, in the right carotid, *r*; the purpose of these will be mentioned presently. Next the first pair of costal cartilages and the piece of sternum between them are resected, artificial respiration started, and the internal mammary arteries found and ligatured where they pass forwards between the apices of the lungs. The sternum and the sternal ends of the ribs are then cut away down to the diaphragm, and if the day is cold a cloth soaked in moderately hot water laid over the posterior half of the chest so as to keep lungs and heart warm, care being taken that it does not touch the pericardium; this hot damp cloth is renewed from time to time as necessary; on a warm day it may be omitted.

Next the superior cava is pushed aside and the right subclavian artery, *w*, clamped and opened. The bulb of a slender thermometer, *a*, is then placed in the vessel and, the clamp being removed, is pushed down into the innominate trunk and tied so as to keep it there. This gives the temperature of the blood flowing through the heart, which cannot be deduced accurately from the temperature of the chamber in which the apparatus is placed; partly because the blood warms and cools more slowly than the air in the box, and partly because in its circuit through the lungs it is cooled. A very small twig given off from the innominate trunk to the anterior mediastinum is also tied. Next the left subclavian, *m*, is isolated and a cannula, *x*, placed in it; and the aortic arch, *l*, tied just beyond the origin of the left subclavian. When the sub-

clavians and aorta are tied (the carotid flow being already stopped)
anæmic or dyspnœic convulsions occur, and arterial pressure rises
very high, as evidenced by the great size to which the stumps
connected with the aortic arch become distended; to obviate this
strain on the heart, the aortic arch is tied as quickly as possible
after putting the cannula in the left subclavian, and before the
dyspnœa is extreme a large quantity of blood drawn off through
the cannula, s, in the right carotid; when what appears sufficient
is drawn the screw-clamp u is tightened up again. Finally the
inferior cava, e, is ligatured, and the azygos vein, f; and a can-
nula, h, put in the superior cava, g. This finishes the operative
procedure.

To get rid of the blood now present in the heart and lungs,
which would be apt to clot in the cannula during a subsequent
prolonged observation, and to replace it by defibrinated blood, of
which about two litres are obtained from other dogs before the ex-
periment, is the next step. The cannula h is filled with whipped
blood and connected with a funnel containing the same warmed to
$35°$ C.; the clamp t on the right carotid is then again opened and
from 300 to 400 c. c. of defibrinated blood run through the heart
and lungs—in by the superior cava and out by the carotid—wash-
ing out and replacing the blood previously present; the blood
drawn is whipped and strained and added to the stock on hand.
The supply should be slow and sent in under a pressure equal
to that exerted by a column of blood about 20 centimetres in
height. The carotid is then again clamped and the vena cava a
second or two later, after the heart and lungs have filled up with
blood. The funnel is now removed and the heart, still lying in
the chest, is ready for transference to the chamber in which it
is to be kept warm and moist and fed with fresh defibrinated
blood.

This chamber consists of a box five feet long, three high, and
two and a half wide. It has no floor; has one wooden end, I; a
wooden back; a glass front; a glass roof, K; and a glass end, L.
The front can be entirely removed and has also a door in it
through which matters can from time to time be arranged inside
and temperatures read off without removing the whole front. The
chamber rests on a galvanized iron trough, DD, which contains
about an inch and a half of water. In it is a Bunsen's regulator
connected with the burners CC, and serving to maintain a uniform

temperature in the interior. In the chamber about an hour before the experiment are placed the glass cylinders 27 and 28, each containing about 800 c. c. of fresh whipped and strained dog's blood, which has thus time to attain the temperature of the interior of the box.

All being ready the front of the chamber is removed and the dog stretcher GG, having on it the dead body of the dog with the living heart and and lungs, is put in. The heart alone is indicated in the diagram to make description of its connections easier. The cylinders 27 and 28 are elevated on a block at the anterior end of the stretcher, so that their lower ends are ten or twelve centimetres above the auricular end of the heart. These cylinders are Marriott's flasks. Each is closed air-tight at the top by a cork through which four tubes pass; one tube in each case (9 and 12 respectively) allows air to enter from the interior of the chamber and reaches to near the bottom; another (5, 6) dips a little deeper into the blood and acts as a syphon to draw it off. The remaining tubes (7 and 10, 8 and 11, respectively,) only reach a short way through the cork. Each has on its upper end a bit of rubber tubing which can be closed air-tight by a clamp, and is so when the cylinder is in use. These short tubes are for filling the reservoirs; when one cylinder is nearly empty, as for instance 27 in the diagram, the clamp, 2, on the tube leading from it to the heart is screwed up, and the communication between the heart and the other reservoir opened; while this second one is feeding the heart the first is refilled by opening the clamps 18 and 17, putting the funnel 19 on the rubber tubing of 11, and refilling the reservoir through it; as the blood enters the air escapes through 10; when the cylinder is filled the clamps 17 and 18 are again screwed tight and the cylinder is again ready for use long before its fellow has emptied.

The syphons leading from each Marriott's flask meet in the Y–piece z from which passes the rubber tubing i. As soon as the animal is placed in the chamber this bit of tubing is filled with blood by opening its connection with one of the reservoirs and is immediately slipped over the end of the cannula, h, in the superior cava, from which the clamp is removed: the heart is thus steadily supplied with blood from each reservoir in turn. The outflow tube, q, passes from the left carotid, o, which is not used for the preliminary bleeding and washing out which, with the object of avoiding any clotting in the left, are done through the right carotid

as above described; now that there is only defibrinated blood to deal with there is no longer any danger of such clotting. Over the cannula, *p*, is slipped one end of the rubber tube, *q*, which leads to the glass tube 21, which passes through the wooden end of the box and has on it a stopcock, 22, beyond which the tube curves round and reënters the box. By means of the stopcock the rate of irrigation can be regulated without opening the chamber; the blood which flows through is received in the vessel 24, which is set aside within the box and replaced by another from time to time as necessary, until one of the Marriott's flasks needs refilling. In this way the blood being nearly always inside the chamber does not get a chance to cool more than a degree or two, and so has ample time to heat up again to the proper point while the other Marriott's flask is emptying. The rate of flow permitted is usually a pretty rapid dropping; but if a low arterial pressure is desired the stop-cock, 22, is opened wider; if a higher it is more closed. Even a slow dropping keeps the heart well alive for a long time; if signs of feebleness come on, all that is needed is to open the stop-cock wide for a few seconds and thoroughly renew the blood in the heart.

Arterial pressure and the pulse curves are obtained from the mercurial manometer 26. This, by means of connecting tubes, filled with sodic carbonate solution in the usual manner, is attached to the cannula *x* in the left subclavian.

All the connections having been made the front is replaced on the chamber and henceforth the heart beats on in it without disturbance, except as from time to time a small door is opened to change the receptacle 24, or take out blood to refill one of the Marriott's flasks and change the one connected with the heart by opening or closing the clamps 1 or 2, or note the temperature of the thermometer *a*.

The description of the various connections to be made after the animal is placed in the chamber takes some time, but the whole thing is done in two or three minutes. While the front of the chamber is out the air in it cools considerably, but the blood of course much less on account of its high specific heat, and in a very few minutes, while one waits for the heart to get uniform and to be sure that brain and spinal cord are dead, all inside is again at a uniform temperature and a series of observations can be commenced. Before commencing these I always wait until all signs of

reflex excitability are lost and the muscles begin to exhibit rigor; this occurs at latest in half an hour after ligaturing the various arteries. Sometimes Traube's curves are seen for a few minutes after the animal is placed in position, shewing that the medulla is not quite dead; but they very soon pass off never to return, though when the heart begins to die something simulating them (to which I will return later) usually occurs..

It is, I think, clear that by this plan of work the study of the physiology of the mammalian heart is made possible to an extent never before attainable; I have now made a considerable number of observations which shew that for at least four hours and often for considerably longer, great regularity and power in the heart's beat can be maintained. I give below in tabular form the successive observations as to pressure in the subclavian and pulse rate made in two experiments, which shew the perfect availability of the method. To investigate the direct action of any drug on the heart one would have only to inject it by a hypodermic syringe into the cardiac end of the tube *i*, as in the usual manner of injecting curari into a vein. By altering the temperature of the chamber one can readily study the effect of various temperatures on the pulse rate, arterial pressure being kept at a given level while the tracings (at intervals of five or ten minutes) are being taken, by altering the outflow through the stopcock, if necessary; between the readings a uniform flow is kept up irrespective of arterial pressure. By keeping the temperature constant and altering the stopcock the direct influence of various arterial pressures on the pulse rate can be readily studied. On these two latter points I have already made a number of interesting observations, which are not, however, yet quite ready for publication. The chemical products of muscular work apart from those eliminated by the lungs must also accumulate in the blood which has flowed round and round the beating heart for hours, and probably can there be examined better than in any other organ at present at our disposal. It seems also to me practicable to unite a given organ, say kidney or liver, with the heart and keep it alive for study, but this I have not yet tried. At any rate it is clear that a large field for investigation of various points of great interest is made available for study under much more favorable circumstances than hitherto.

When the heart begins to die the first symptom is an irregular rhythm which cannot be removed by free irrigation with the blood

in the reservoirs. Whether this is immediately due to changes in
the heart itself, or to the consumption of food materials in the
stock of blood, or to the accumulation in it of wastes usually
removed by the kidneys or other organs I cannot at present state.
Whether it be due to the first of the above causes could readily be
decided by taking an entirely fresh stock of defibrinated blood.
The irregularity manifests itself by a large beat followed by three
or four smaller ones, and so on for more than an hour. Then the
small beats become feebler and feebler, and, arterial pressure being
consequently very low, the pulse due to the more powerful beat
very conspicuous. Finally the large beats alone remain, and they
gradually become less and less until they disappear. In its earlier
stages the phenomenon has an interesting resemblance to the sec-
ondary rhythm observed in the frog's heart under certain circum-
stances; it is what I referred to above in stating that late in the
experiment something simulating Traube's curves is often seen.

For the guidance of those who may repeat the experiment, I
may add that the thing most to be avoided is sending blood into
the superior cava too fast or under too high a pressure; this is far
more fatal than considerable cooling or delay.

The following tables give the results of two experiments. In
each case the number indicated in the column headed "pressure"
is the pressure in millimetres of mercury indicated by the mano-
meter connected with the left subclavian artery. The numbers in
the column headed "pulse" give the number of heart beats per
minute. Temperatures, when given, (Table II.) are not accurately
those of the heart or blood, but those of the chamber in which the
heart lay. The introduction of a thermometer into the innomi-
nate trunk is one which I have only used in later experiments on
the influence of temperature changes on the pulse rate, when an
accurate knowledge of temperature was essential; in the experi-
ments given here the point I had in view was merely to determine
whether an isolated heart could be kept alive long enough for
study; and accuracy as regards temperature readings within a de-
gree or two was not essential.

Table I records the first experiment, which showed me that the
end I had in view was really attainable, and is given partly, per-
haps, because I have a special interest in it on that account, but
chiefly because it illustrates how well the heart will live under
very rough experimental conditions. At the time when it was

made I had not arranged any warm chamber, and the heart was simply warmed in the roughest manner by inverting a tin pan over the body of the dog and putting a Bunsen's burner under this; with some wet cloths to keep the atmosphere moist. From time to time, the gas was turned down or up as I thought the temperature round the heart was too high or too low, but no thermometer readings were taken, and the temperature no doubt varied very much in the course of the experiment. At this time also the use of the Marriott's flasks had not been thought of: from time to time, as the heart seemed weakening, fifty cubic centimetres of whipped blood were run in by the vena cava and an approximately equal bulk removed through the carotid. The numbers given therefore as to pulse rate and arterial pressure have little or no value; and the whole experiment simply serves to show with what rude appliances the isolated heart can be kept at work for a long time when the coronary circulation is maintained.

Table I.

Experiment of April 1, 1881.

Time. P. M.	Pressure.	Pulse.	Remarks.
1 h. 35′.			Finished tying up all the vessels but those of the pulmonary and coronary circuits.
1 h. 40′.	68	96	
2 h. 20′.	74	87	
2 h. 22′.			Fresh blood run in.
2 h. 23′.	96	104	
2 h. 30′.	93	102	
2 h. 37′.	118	96	
2 h. 40′.	80	93	
2 h. 50′.	96	100	
3 h. 04′.	60	100	Fresh blood run in at 3 h. 3′.
3 h. 21′.	86	96	
3 h. 28′.	104	42	Cold blood run in at 3 h. 27′.
3 h. 50′.	32	96	
3 h. 51′.			Fresh warm blood run through.
3 h. 52′.	92	112	
4 h. 06′.	41	88	
4 h. 13′.	25	80	
4 h. 15′.			Fresh warm blood run through.
4 h. 16′.	92	86	

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Table I.—*Continued.*

Time. P. M.	Pressure.	Pulse.	Remarks.
4 h. 29′.			Fresh warm blood run through.
4 h. 30′.	92	79	
4 h. 39′.			Fresh warm blood run through.
4 h. 40′.	90	88	
4 h. 47′.	56	88	
4 h. 59′.			Fresh warm blood run through.
5 h. 00′.	76	86	
5 h. 09′.	43	96	
5 h. 10′.			Fresh blood taken from another dog and not used before in the course of this experiment, run through.
5 h. 18′.	140	88	
5 h. 23′.	58	72	
5 h. 26′.			Fresh blood.
5 h. 29′.	116	83	
5 h. 33′.	52	76	
5 h. 35′.			Fresh blood.
5 h. 40′.	60	82	
5 h. 44′.			Fresh blood.
5 h. 45′.	102		Chronograph pen out of order, so the pulse rate cannot be given.
5 h. 48′.	76		
5 h. 53′.			Fresh blood.
5 h. 55′.	92	92	
6 h. 00′.	37	88	
6 h. 02′.			Fresh blood.
6 h. 03′.	61	98	
6 h. 11′.			Fresh blood.
6 h. 14′.	88	92	
6 h. 20′.	42	88	
6 h. 22′.			Fresh blood run in; none drawn off.
6 h. 24′.	118	98	
6 h. 30′.	32	97	
6 h. 35′.	24	96	
6 h. 36′.			Fresh blood run in; none drawn off.
6 h. 38′.	118	100	
6 h. 41′.	28	84	
			The beat immediately afterwards became very irregular, and ceased finally at 7 h. 10′.

The above experiment, as already stated, justifies no conclusions except that an isolated mammalian heart can be kept beating for several hours. It, however, suggests (and subsequent experiments, which I hope shortly to publish, confirm) that the pulse rate of the isolated heart is very independent of arterial pressure, though, as no accurate temperature observations were made in this case, the experiment by itself is not worth much in that respect.

Table II.

EXPERIMENT OF MAY 26, 1881.

Time. P. M.	Temp. in degrees C°	Pressure.	Pulse.	Notes.
1 h. 50′.				All vessels tied but those of the coronary and pulmonary circuits. Then 150 c. c. of warm whipped blood sent through the heart in order to wash out the blood already in it and in the lungs.
2 h. 05′.				Animal removed to warm chamber and the irrigation started from the Marriott's flasks and maintained thenceforth.
2 h. 15′.		72		Pulse rate not known, as the chronograph was not working.
2 h. 45′.	95°	72	92	
3 h. 00′.	99°	86	118	
3 h. 15′.	98°	87	118	
3 h. 55′.	99°	90	120	
4 h. 15′.	99°	91	120	
4 h. 35′.	100°	87	118	
4 h. 50′.	100°	86	120	
5 h. 10′.	100°	68	117	
5 h. 45′.	100°	64	117	
6 h. 00′.	100°	60	118	
6 h. 15′.	99°	56	117	Arterial pressure now began to fall markedly, and while a fresh supply of blood was being obtained from another dog (that in use having already circulated round the heart many times, and being presumably full of wastes) the organ ceased to beat at 6 h. 45′.

The experiment described in Table II was made in the warm chamber described in the preceding pages and with the Marriott's flasks, giving a uniform instead of the intermittent supply of fresh blood used in the experiment of Table I. It is one of a number which all shew the great regularity which can be obtained for some hours in the heart's work under such circumstances; and hence the possibility of readily observing the influence on its activity of various conditions and of drugs: in other words, it indicates that the separated organ is in a fit condition for physiological or therapeutical experiment.

During the earlier part of the above experiment (from 2.15 to 3.00 P. M.) the chamber and its contents were considerably cooled in consequence of one of the Marriott's flasks being out of order and necessitating the keeping open of the doors, for its repair. When this was accomplished, we find for the subsequent two hours (3 h. 00′ to 4 h. 50′) a very remarkable uniformity in the heart's work. Arterial pressure only varies between 86 and 91 mm. of mercury, and the pulse rate between 118 and 120 per minute. Probably under no conditions would a heart still connected physiologically with the rest of the body display so great a uniformity in its activity for so long a time. The pulse, it will be seen, still remained very regular to the end of the experiment, although arterial pressure fell; this again illustrates the slight influence exerted by aortic pressure upon the rhythm of the isolated heart.

A NOTE ON THE PROCESSES CONCERNED IN THE SECRETION OF THE PEPSIN-FORMING GLANDS OF THE FROG.

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It has been shown, chiefly through the labors of Langley, that the œsophageal glands of the frog undergo in digestion marked histological changes. When the animal in healthy condition has fasted several days, the œsophageal glands are full of fine granules throughout, and no boundary lines between the cells can be made out in the fresh gland. Examined two or three hours after feeding the glands are found to be void of granules on their outer borders, the hyaline matrix alone remaining; this process may extend until all the granules have disappeared except a larger or smaller group collected round the gland lumen. The return to the normal resting appearance occurs usually one to two days after light feeding. These changes are in certain species, as in *R. temporaria*, so well marked that I thought it advantageous to use them as a sign of the secretory activity of the œsophageal or pepsin-forming glands, when these were excited by the absorption of different food materials from other than the usual surfaces. It was thought that one might thus get a better idea of the conditions and mechanism of secretion. The experiments were conducted in the cold months, from February to May, and the frogs were in large part taken fresh from the mud in which they had buried themselves for the winter. The great changes necessarily taking place in the animals while coming into a more active condition, no doubt account for much of the want of uniformity which was observed in the behavior of many of the specimens examined. The animals chiefly used were small specimens of the bull-frog, *R. mugiens.* Of two frogs apparently alike it sometimes happened that one would show distinct secretory changes in the œsophageal glands three hours after feeding on meat or beef fibrin, while the other examined at the same time preserved the hungering appearance of its glands unchanged, but this was exceptional.

In a small spotted frog, *R. halecina*, the diminution of granules in digestion, if it occurs at all, goes on very slowly. I was not

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able to detect a disappearance of the granules in this frog in less than twenty-four hours after feeding, though digestion had evidently been active in the interval. A conclusion may be stated in advance that secretion apparently involves the glands simultaneously in two opposite activities, a breaking down and a building up, and it is the ratio of the vigor of these changes which determines the histological appearance of the gland at any time. Nearly all the microscopic examinations were made upon the fresh gland by snipping off a piece of the mucous membrane of the œsophagus and mounting quickly in iodized amniotic fluid of the sheep.

For convenience in description I will indicate by letters the various solutions used as food stimuli. *A* was a solution obtained by extracting beef muscle with 0.5 per cent. NaCl. *B* was a concentrated commercial "peptone," said to be the product of the peptic digestion of beef muscle; this was diluted five to ten times before use; it contained probably the flesh "extractives" as well as peptone. *C* was a concentrated solution obtained by boiling dog's muscle in water. *D* was a strong watery solution of peptone, made in the laboratory by the peptic digestion of fibrin. *E* was 0.5 per cent. NaCl.

In all cases comparative examinations were made of similar frogs unmolested and experimented upon.

The fluids mentioned were injected in quantities usually of 1 to 2 c. c., either into the rectum or under the skin.

When the injection was into the rectum the fluid was usually allowed to flow from a pipette inserted into the anus; a safer way was subsequently found in gently tying the frog in a prone position and allowing the solution to run from a burette through a cannula into the rectum. There was no evidence in any case of fluid having reached the stomach. The bladder and rectum only appeared to be filled.

1. *Injection into the rectum.*

Injection of both *A* and *B* into the rectum caused in the hungry frog marked disappearance of granules from the œsophageal glands. The evidence as to *C* was not satisfactory. *E* appeared also to have a distinct effect. It is to be remarked that the disappearance of granules begins very quickly after the injection, the process is rapid and recovery to the original condition of full granulation is speedy, except in the case of injection with NaCl. The diminution of granules is marked twenty minutes after injection, and in

fifty to eighty minutes the glands have again become granular throughout.

The stomach of a frog which has hungered several days contains generally very little mucous fluid, which is usually acid. The stomach wall itself appears to be always acid. In the experiments described above there was no considerable increase in the stomach contents accompanying the histological change of the œsophagus. And it may be said here that I have' been able to discover no relation between the amount of fluid secreted into the stomach during any period and the histological appearance of the œsophageal glands at that time.

2. *Injection into the dorsal lymph sac.*

There was found no distinct evidence of a diminution of granules following a hypodermic injection of the fluids enumerated above. On the contrary, the injection of *B* was almost always succeeded by an accumulation of granules in the glands, even under conditions, as in active digestion, in which a diminution of granules was to have been expected. Such an injection apparently accelerates and intensifies the normal digestion.

3. *The relation between the injection into the lymph sac and the amount of secretion found in the stomach.*

When *C* was injected under the skin of a fasting frog, the stomach was found at the end of one to two hours very much distended, with a rather thin, neutral or slightly acid, mucous fluid. The same result to somewhat less extent followed the use of *D*. This effect from *B* was particularly marked where the frog had been previously fed. The results from *A* were in the same direction but less noticeable, and no such effect followed the use of *E*. No experiments were made to determine the digestive value of this secretion. It appeared to increase in quantity for a period considerably longer than that required by the artificially excited glands to recover their resting appearance. The causes that produce the secretion seem rather to increase than diminish the granules of the glands.

As to what are the steps in the recovery of granules by the glands after their disappearance in digestion, nothing decisive can be said. There was one well marked histological character which distinguished the glands of certain frogs, the meaning of which seems well worth investigation.

This peculiarity was the presence of very large well defined masses in the gland cells, usually in their outer part. These masses were strongly suggestive of some of the forms of lymph corpuscles which are numerous in the wall of the œsophagus. They have much the refractive characters of the fat or of fresh fat cells. Sometimes they exist as clumps of highly refractile granules, and it is clear that their substance exists in very different states of division in the glands.

These bodies stain black with osmic acid. They are dissolved by ether. This reagent dissolves out also all of the granules from the œsophageal glands, leaving behind only a clear gland substance with the cell nuclei imbedded therein. It may be observed that it is unsafe to draw conclusions from the appearance of specimens preserved in balsam, for the preliminary treatment with the clearing fluid dissolves out many of the finer granules previously present in the cells.

The granule masses referred to were by far most numerous in glands which, there was reason to think, were being actively regenerated as to their granules; that is after a long period of normal digestion or in the case of the injection of a food solution, as *B*, during normal digestion. The reactions indicate that they are of fatty nature.

General conclusions:

The general conclusions toward which these results lead are that the secretory changes in the glands of the œsophagus are started by the mere absorption of matter from the alimentary canal but that the regeneration of the glands depends upon the presence of new matter in the blood itself.

The presence of foreign matter in the blood may cause an extensive secretion into the stomach and if in this case the secretion comes from the œsophageal glands these are rebuilt quite as fast as they are broken down. It is probable that the secretory process is initiated by stimuli in the alimentary canal but is chiefly carried out by the influence of substances newly absorbed into the blood.

The secretion of the acid of the gastric juice seems to be due to the presence of food matter resting in and absorbed by the stomach itself. The results obtained from these experiments give general support to Schiff's views concerning peptogenic substances.

LIST OF MEDUSÆ FOUND AT BEAUFORT, N. C., DURING THE SUMMERS OF 1880 AND 1881. By W. K. BROOKS.

DURING the two seasons which we have spent at Beaufort the members of our party have derived great benefit from the lists of the Vertebrate and Invertebrate fauna of Fort Macon, by Drs. Yarrow and Coues, which were published by the Philadelphia Academy of Sciences in 1871. We have been able to make many additions to these lists, especially in the various groups of invertebrates, and as the authors made no attempt to collect or identify the medusæ of these waters I have drawn up the following list of Acalephs and Ctenophorae, from the notes which I made during the summers of 1880 and 1881.

McCrady and L. Agassiz have studied the medusæ of South Carolina, and I give, for convenience of reference, a list, compiled from these authors by A. Agassiz (N. A. Acalephs), of the forms which occur at Charleston, for comparison with my own list of Beaufort species.

Acalephs of Charleston, S. C. (From A. Agassiz' *N. A. Acalephs*, pp. 223–4.)	Acalephs Collected at Beaufort during the Summers of 1880 and 1881.
Bolena littoralis, McCr.	
Mnemiopsis Gardeni, Ag.	*Mnemiopsis Gardeni*, Ag.
	Mnemiopsis Leidyi, A. Ag.
Beroe punctata, Esch.	
Idyiopsis Clarkii, Ag.	*Idyiopsis Clarkii*, Ag.
Stomolophus meleagris, Ag.	(1.) *Stomolophus meleagris*.
	(2.) *Dactylometra quinquecirra*, Ag.
Cyanea versicolor, Ag.	
Foveola octonaria, A. Ag.	(3.) *Foveola octonaria*, A. Ag.
	(4.) *Cunina discoides*, Fewkes.
	(5.) *Cheiropsalamus quadrumanus*, F. Müller.
	(6.) *Tamoya haplonema*, F. Müller.
Persa incolorata, McCr.	(7.) *Persa incolorata*, McCr.
Liriope scutigera, McCr.	(8.) *Liriope scutigera*, McCr.
	(8.) *Liriope scutigera*, A. Ag.
Oceania folliata, Ag.	(9.) *Oceania folliata*, Ag.
Eucheilota ventricularis, McCr.	(10.) *Eucheilota ventricularis*, McCr.
	(11.) *Dipleuron parvum*, sp. nv.

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Clytia bicophora, Ag.
Platypyxis cylindrica, Ag.
Eucope divaricata, A. Ag.

(9.) Campanularia noliformis, McCr.

Obelia commisuralis, McCr.
Eirene gibbosa, Ag.
Eutima mira, McCr.

(12.) Eucopa obliqua, sp. nv.
      Obelia commisuralis, McCr.
      Eirene gibbosa, Ag.
      Eutima mira, McCr.
(13.) Eutima cuculata, sp. nv.
(14.) Eutima emarginata, sp. nv.

Eutima variabilis, McCr.
Aglaophenia tricuspis, Ag.
Aglaophenia trifida.

(15.) Nematophorus, sp. nv.

Plumularia quadridens, McCr.
Plumularia (Catharina-like), McCr.
Dynamena cornicina, McCr.

(16.) Dynamena bilatteralis, sp. nv.

Diphasia (nigra-like), Ag.
Margelis Carolinensis, Ag.
Nemopsis Bachei, Ag.
Eudendrium ramosum, McCr.
Turritopsis nutricula, McCr.
Stomatoca apacata, McCr.
Willia ornata, McCr.
Dipurina cervicata, McCr.
Dipurina strangulata, McCr.
Corynetis Agassizii, McCr.
Gemmaria gemmosa, McCr.
Pennaria tiarella, McCr.

(17.) Margelis Carolinensis, Ag.
(18.) Nemopsis Bachei, Ag.
      Eudendrium ramosum, McCr.
(19.) Turritopsis nutricula, McCr.
(20.) Stomatoca apacata, McCr.
(21.) Willia ornata, McCr.

      Dipurina strangulata, McCr.
      Corynetis Agassizii, McCr.

      Pennaria tiarella, McCr.
(22.) Pennaria inornata, sp. nv.

Ectopleura turricula, Ag.

      Ectopleura ochracea, A. Ag.

Parypha cristata, Ag.

      Parypha cristata, Ag.
(23.) Steenstrupia gracilis, sp. nv.

Hydractinia polyclina, Ag.
Eudoxia alata, McCr.
Diphyes pusilla, McCr.
Physalia arethusa, Til.
Velella mutica, Bosc.
Porpita linniana, Less.

(24.) Hydractinia polyclina, Ag.
      Eudoxia alata, McCr.
      Diphyes pusilla, McCr.
      Physalia arethusa, Til.

      Porpita linniana, Less.
      Nanomia cara, A. Ag.

(1.)   Stomolophus meleagris, Ag.

We found no living specimens of this species in 1880, although
the remains of two or three were found on the sand bars at low
tide, early in June.

In June, 1881, living specimens were extremely abundant both
outside the bar and in the sounds.

They could be seen floating or swimming at the surface on all sides of the boat, and although they were so shy that they sunk when approached, they were so abundant that we easily captured all we could carry home. Those which we secured were from four inches to twelve inches across the opening of the umbrella, although larger specimens were seen.

Later in the season they were less abundant, but we found specimens occasionally through June, July and August.

The fact that such a large and conspicuous species should be so abundant one year and almost absent another year shows the impossibility of thoroughly studying the fauna of our coast without permanent marine stations.

(2.) *Dactylometra quinquecirra*, Ag.

This medusa is found in abundance all through the summer in the lower part of the Chesapeake Bay. We never found it inside the inlet at Beaufort, although we occasionally found it just outside the bar, and early in September, 1880, it was common.

The southern form swims at the surface at all hours of the day and night, and as it differs from A. Agassiz' description in several slight particulars, it is probably a well marked southern variety.

(3.) *Foveola octonaria*, A. Ag.

Rather abundant in June and early July. Although *Turritopsis nutricula* is our most common medusa, we never found the young Cunina in its bell.

(4.) *Cunina discoides*, Fewkes.

In August, 1880, I procured a single mutilated specimen which is very similar in general form to Fewkes figure of *Cunina discoides*, although it has but twelve tentacles, and eight sense organs.

(5.) *Cheiropsalamus quadrumanus*, F. Müller.

This interesting medusa will probably be found to be by no means rare along our coast, although it is seldom found at the surface.

McCrady has found one specimen at Charleston, and one at Port Royal.

In July, 1880, we found a few specimens on the sand bars at low tide, and throughout July, August and September we got specimens in from three to eight fathoms outside the bar, on sandy bottom. The specimens were taken from the bottom with the trawl, and we found none at the surface, although those which we kept in aquaria in the house swam near the surface. They were from one inch to five inches across the umbrella.

(6.)   *Tamoya haplonema*, Fr. Müller.

In July, 1880, a fisherman brought me a single living female of this species. We found no others.

(7.)   *Persa incolorata*, McCr.

Found occasionally at night, swimming at the surface, from June 24th to August 8th. It is a very delicate species but many of our specimens were perfect and healthy. We found twenty or thirty in all. It is one of our most rare medusæ. McCrady found four specimens, and Haeckel has found other species of the genus, but it seems to have entirely escaped other observers.

(8.)   *Liriope scutigera*, McCr.

McCrady's *Liriope scutigera* is one of the most common medusæ at Beaufort, and as we found specimens at all stages of growth, we were able to trace the whole of the interesting metamorphosis, and to decide that it is not the same as *L. scutigera*, A. Ag. A single specimen which seemed to belong to the latter species, was found in July, 1880.

(9.)   *Oceania folliata*, Ag.

We were able to trace the whole life-history of this abundant species, and to settle a number of doubtful points concerning it.

The hydra—*Campanularia noliformis*, McCr.—is very like Agassiz' *Platypyxis cylindrica*, but may be distinguished from it by several constant features.

The upper or distal end of the reproductive calyx, is truncated squarely instead of flaring, and the outline of the calyx is alike in side and front view.

The four or five medusæ which it contains are nearly equal in size, and they are discharged in quick succession, the last escaping within a few minutes after the first.

The medusa, *Epenthesis folliata*, McCr. is very similar to *Oceania languida*, A. Ag., but the tentacles and otocysts develop as A. Agassiz describes them in *Clytia bicophora*, Ag.

The difference between the hydra and *Platypyxis cylindrica*, is so slight that a thorough knowledge of the life history of the latter may show that it is only a northern variety; but there can be no question as to the specific distinctness of the medusa from *Oceania languida*.

(10.)   *Eucheilota ventricularis*, McCr.

Mature and nearly mature medusæ are common at Beaufort, from July 15th to the end of August, but the young ones were more rare, although I was able to get a sufficiently complete series to show that the young medusa found at Naushon, by Alex. Agassiz, undoubtedly belongs to this species.

(11.)   *Dipleuron*, novum genus.

Medusa with four radiating chymiferous tubes, four radial tentacles with basal cirri, and twelve otocysts, four interradial and eight on the sides of bases of radial tentacles. Reproductive organs two, nearly spherical, on two opposite chymiferous tubes, near bell margin. Stomach short, with simple mouth, without oral tentacles.

Dipleuron parvum, sp. nv.

Umbrella nearly as high as wide in profile view, with greatest transverse diameter about half-way up, where there is a distinct angle in the outline. Umbrella of uniform thickness from top to free edge; elliptical when seen from above or below, with major axis nearly twice as long as minor axis. Proboscis a little enlarged at the circular mouth, which has a simple edge. Reproductive organs spherical, two in number, on two opposite radiating tubes near bell margin, with a large central chamber, opening into radiating tube by a long narrow vertical slit.

The four radial tentacles are usually carried with their tips turned upwards. Each tentacle carries at its base two small

twisted cirri, and consists of a swollen pigmented bulb which passes gradually into a long slender filament, which is usually coiled in a loose spiral.

Otocysts twelve in number, of two kinds; four large ones half-way between the tentacles, and eight smaller ones, two at the base of each tentacle. Each otocyst has a single otolith, and the small otocysts are sometimes absent.

The largest specimens are about $\frac{166}{1000}$ inch in longest diameter.

This species is common at Beaufort, from June 5th to August 8th, and sexually mature specimens of both sexes are frequently found.

It somewhat resembles A. Agassiz' *Eucheilota duodecimalis,* (*Phialium dodecasemum,* Haeckel,) except that the reproductive organs are always two, and spherical.

(12.) *Eucope obliqua,* sp. nv.

Communities from half to two-thirds of an inch high. Hydrotheca slightly flaring at edge. Knee oblique, lowest on side nearest main stem, and highest on outside. Stem with from five to seven annulations above each fork. Hydranths colorless, with about thirty tentacles so placed that their tips form two circlets.

Reproductive calycles long, nearly cylindrical, abruptly truncated at tip.

Medusæ arranged in two rows; seven or eight maturing together.

When discharged the medusa is about $\frac{8}{1000}$ inch across disc, with two otocysts and six or seven tentacles in each quadrant.

The hydræ were frequently found on floating pieces of Sargassum and on drift wood. The number of tentacles at the time the medusa escapes from the calycle is quite variable, and although twenty-four seems to be the normal number, I did not find a single specimen with exactly twenty-four. Usually three of the quadrants had six each, and the fourth seven or sometimes five.

After the escape of the medusæ the distal half of the calycle falls off, and its proximal end becomes converted into an ordinary hydrotheca.

(13.) *Eutima cuculata,* sp. nv.

Umbrella flat: height about one-fourth diameter. Gelatinous substance very thick in centre, so that the cavity of the sub-um-

brella is very shallow, and makes less than half the total height of bell.

The umbrella diminishes in thickness gradually towards the bell margin, where it forms a thin edge. Prolongation into proboscis conical above, prismatic below, more than twice as long as height of umbrella. Stomach a little enlarged, forming about one-fifth of total length of proboscis, with four simple lips. Four radial tentacles, very long, slender, imperfectly retractile, with very slight basal enlargements, without accessory cirri. Nine or ten slight enlargements of circular tube in each quadrant, and a few of the enlargements have accessory cirri. Two otocysts, with from three to eight otoliths, in each quadrant.

Reproductive organs run along radiating tubes from circular tube to conical part of proboscis, but they do not run down onto prismatic portion.

About one third of an inch in diameter. Stomach and tentacular bulbs intense green by reflected light; ectoderm of tentacular bulbs sky-blue, and endoderm bright pink by transmitted light.

A few specimens were found August 7, 1880. The bases of the tentacles are covered by small semicircular flaps or hoods, from the gelatinous substance of the bell, and I have named the species from these, although similar hoods are found in *Eutima mira*, McCr.

The species may readily be distinguished by its very flat disc-like umbrella, and by the great length of the tentacles. When these were thrown out to three times the diameter of the bell they were far from straight, but were thrown into a number of sharp angular zig zag folds. At first sight this species might seem to belong to Haeckel's *genus Eutimium.* Although the basal cirri are entirely absent, careful examination shows that the marginal enlargements and cirri are present, but very small.

(14.) *Eutima emarginata*, sp. nv.

During the summer we occasionally found specimens of what seems to be another new species of *Eutima*, but a more complete knowledge of its life history may possibly show that it is the young of a described species. If so it must undergo considerable metamorphosis.

It may be described as follows:

Medusa with a rather low bell, one third as high as wide, with a strongly emarginated rim. Gastrostyle about three times as long

as height of bells, prismatic, with four prominent ridges along the radiating tubes. Stomach no wider than, and about one third as long as style, with four simple lips. Radiating tubes enlarged to form four fusiform chambers on lower end of style, just before they join stomach. Four radiating tentacles, tapering gradually from base to tip, and capable of almost perfect retraction, although they are never extended much further than the length of the proboscis.

Two otocysts with three ossicles each, in each quadrant. From ten to twelve enlargements and three or four cirri in each inter-radius, and a cirrus on each side of base of each radial tentacle.

The reproductive organs were not observed.

No hoods over radial tentacles.

The largest specimens were about one third of an inch in diameter.

(15.)   *Nematophorus*, sp. nv.

On August 18th, 1880, we took with the trawl off Fort Macon, in three fathoms of water, great quantities of a beautiful feather-like hydroid community, the stems being a foot or more in height. They were all torn away from their attachment, but there was no way to decide whether they had been pulled up by the trawl or were drifting specimens from a distance.

The hydranths were alive, but they soon died in confinement, and I did not see any in an expanded state.

At the base of each pinna there is one of the rounded perforated bodies upon which Clarke has founded the *genus Nematophorus*, but our species is much more like a typical *Aglaophenia* than Clarke's *Nematophorus grandis*, and I cannot, without specimens for comparison, state positively that it is not one of the described species of *Aglaophenia*.

(16.)   *Dynamena bilatteralis*, sp. nv.

Stems simple, unbranded, slightly curved; from one-fourth of an inch to one inch high; springing from a creeping hydrocaulus. From five to twenty pairs of hydranths on each stem. Hydrothecæ long, in contact with each other along middle line of convex side of stem for about two-thirds of their total length. The distal third bends outwards almost at right angles, and the bilobed openings are almost parallel to the stem.

The tentacles of the hydranth are arranged in an ellipse, with its long axis at right angles to the long axis of the stem. The tentacles at the ends of this axis are the shortest, and those at the ends of the minor axis, or the top and bottom tentacles, are the shortest. Tentacles about twenty-two. Reproductive calycles at base of stem, nearly spherical, with two or three obscure annulations, a short constricted stalk, and a small circular mouth.

This form bears a general resemblance to *Dynamena cornicina,* but I have never seen anything like the horn-shaped reproductive calycles which he describes.

It is very abundant at Beaufort all through the summer. When kept in confinement in a small quantity of water, the tips of the stems grew to a length of several inches, forming a slender transparent spiral thread. When the tips of these threads come into contact with the sides of the glass, they become attached, and throwing out branches, become the hydrorhizae of new communities, which flourish after the parent stock has died.

(17.)   *Margelis Carolinensis,* Ag.

Very common all through the summer, but we did not find the hydra.

(18.)   *Nemopsis Bachei.*

A few specimens were found in the early spring of each season. The Beaufort form seems to be a southern variety, for all the specimens found differ from A. Agassiz' figure, and from sketches which I made in 1874 in his laboratory at Newport, in the outline of the bell, and in the form of the median radial tentacles. The bell is more flattened and its diameter exceeds its height, and the median tentacles have rather slender shafts, with abrupt enlargements at their tips.

(19.)   *Turritopsis nutricula,* McCr.

This medusa is found all through the season, and is the most common species at Beaufort.

The young stages figured by A. Agassiz do not belong to this species.

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Notwithstanding McCrady's excellent description and figures, Fewkes has figured and described it as a new genus and species *Modeeria multitenticulata.*

(20.) *Stomatoca apacta*, McCr.

Rather common at Beaufort all through the summer.

(21.) *Willia ornata*, McCr.

This is a rare species at Beaufort, and I have not met with any sexually matured specimens. Those I found were obtained on July 12th and 13th and August 18th, 1880.

The largest specimens had four stolons running off from the four corners of the stomach just below the inner ends of the radiating tubes. Each stolon soon branched dichotomously, and ended in a medusa bud.

(22.) *Pennaria inornata*, novum species.

Stems wiry, horn-colored, branching irregularly so as to build up a loose arborescent tuft five or six inches high. Hydranths irregularly placed, usually on short lateral branches from secondary stems, sometimes on tips of secondary stems, and occasionally on short branches which spring directly from sides of large trunks.

Stem has from five to seven annulations distal to each fork, and an equal number proximal to each hydranth.

Hydranths nearly colorless, with a circlet of from ten to twenty short tentacles—only one-third as long as hydranth—near the base, and three, or sometimes only two, circlets of short clavate tentacles around the long slender manubrium. There are usually five of the clavate tentacles in the distal set, more in the second set, while the proximal set varies greatly and may be absent.

Taken with the trawl outside Fort Macon, August 18th, 1880.

(23.) *Steenstrupia gracilis*, novum species.

Umbrella bell shaped, circular in cross section, with a long, conical, sharply pointed apex, which makes half the total length, and contains a still longer undulating prolongation from the stomach. One long tentacle and three rudimentary ones, one longer than the other two, and all four without ocelli. The long

tentacle—the dorsal tentacle of Haeckel—may be extended to nearly twice the length of the umbrella including the apex; it is ringed, and ends in a spherical enlargement. The bulb at its base is no larger than those of the other three tentacles, and it has no ocellus.

The tentacle opposite it—the ventral tentacle of Haeckel,—is about three times as long as the other two rudimentary tentacles, and the length of these latter is about equal to their width.

Radiating tubes arch upwards a little from the stomach, and then pass outwards and downwards in graceful curves to the circular tube.

Stomach usually about three-fourths as long as the cavity of the sub-umbrella, although it may be protruded from the opening. It is a little swollen in the middle, and tapers gently towards each end.

The sides of the umbrella are nearly uniformly thick from top to bottom, and in profile view their outline passes into that of the apex by a very gentle curve, which is first convex and then concave.

Length of apex $\frac{3}{16}$ inch, height of umbrella $\frac{3}{16}$ inch, ordinary length of long tentacle about $\frac{3}{8}$ inch.

Found only on June 20th, 1880, in Newport River.

This graceful medusa may readily be distinguished from *Corymorpha pendule*, Ag., by the elongated apex, as well as by the fact that the longest of the rudimentary tentacles is opposite the long tentacle.

It may be distinguished from *Hybocodon* by its circular outline in cross section.

On August 2d, 1880, and on two or three days of the same week, I took from muddy bottom in three or four feet of water, a number of specimens of a solitary hydroid, which may be the young hydra of this species.

The specimens were naked, about $\frac{1}{3}$ inch long, and they had at the upper end of the long slender body, a circlet of about twelve long slender tentacles, with pigment spots at their tips; and some distance above these, a circlet of six short clavate tentacles, also pigmented at their tips.

The animals fastened themselves to the bottom of the tumbler in which they were kept, and I was able to change the water without disturbing them. The lower end of the body soon became encased in a sheath of grains of sand and other small particles.

They multiplied by transverse fission, the upper end separating off, and fastening itself to the glass near the old trunk, which soon developed a new head.

(24.)   *Hydractinia polyclina,* Ag.

The Beaufort Hydractinia is quite different from the descriptions of the northern form, but I made no careful examination of it.

Summary.

Alex. Agassiz includes *Velella mutica* among the Charleston species on the authority of McCrady, but as McCrady only says, " I have never seen a *Velella,*" we may omit it, and we shall then have 42 species recorded as occurring at Charleston, and 43 found in two summers at Beaufort. Of these 43, 27 or more than half occur at Charleston, and eight of the remaining 16 are new.

This list is not complete as there are three or four other forms which are not described at present, as the data are insufficient, and as our stay at Beaufort included the summer months there are, no doubt, a number of winter species which we have not obtained.

Our open boat was so ill adapted for facing the line of breakers on the bar that it was seldom safe to venture outside for a prolonged excursion, so we did very little with the deep water forms, which our short excursions lead us to believe are very numerous and interesting.

November 9th, 1881.

ON THE ORIGIN OF THE SO-CALLED "TEST-CELLS" IN THE ASCIDIAN OVUM.

By J. PLAY-FAIR McMURRICH, B. A., *Assistant in the Biological Laboratory, University of Toronto.* With Plate X.

THE following observations have been made in the hope of elucidating to some extent the nature of the so-called "test-cells," so characteristic of the ova of Tunicates. These bodies have been described by various authors as occurring in the eggs of most of the commoner forms, and under normal circumstances probably do not make their appearance until after fertilization. Lacaze-Duthiers[1] states that in Molgula a true layer of "test-cells" is wanting, and only the follicle-epithelium surrounds the newly deposited ovum. Under abnormal circumstances, however, they are formed at a much earlier period, and thus in most eggs that have been observed, "test-cells" were present or soon made their appearance.

My observations have been carried on for the most part on ova of *Ascidia amphora*, but I have also confirmed most of them by similar experiments on eggs of *Cynthia ocellata*. I made use only of mature or almost mature eggs, so that I am unable to give as complete an account of some points as could be desired.

The mature eggs of *A. amphora* (Pl. X, Fig. 1) have an average diameter of about .255 mm. and present on optical section two distinctly marked zones, enclosing a semi-transparent granular mass, the yolk. The outer of the two zones is formed by the follicle-epithelium, consisting of a single layer of cells surrounding the whole egg, and presenting on a surface view a polygonal appearance. On examining a single cell which has become separated from the egg, with a rather high magnifying power, its interior is seen to be occupied almost entirely by a number of vacuoles, separated from one another and surrounded by very delicate bands of protoplasm, which, in some of the angles formed by the meeting of the polygonal vacuoles, appear as dark spots. (Pl. X, Fig. 2.) I have not been able to observe the development of these cells, but Semper[2] has described it as it occurs in *Molgula nana*, where, in

the earliest observed stages, they appear as a layer of flat cells on the surface of the egg, which, later on, become prismatic, and in the interior of which 2–4 yellow granules make their appearance. These afterward disappear and large vacuoles take their place, pressing the protoplasm and nucleus to one side. In his figures, the formation of the vacuoles has not advanced as far as in the eggs I studied, but, on comparing his Figure 5, Plate I, with my Figure 2, Plate X, it will at once be recognized that the appearance I have observed is to be accounted for in the same manner, the vacuoles having become exceedingly abundant, and pressed the original contents of the cell to the periphery, small portions only being left in the intervals between the vacuoles.

Fol,[3] having succeeded in tracing the origin of these cells still farther back in *Phallusia intestinalis*, states that they are not formed from the ovary, but from the interior of the egg at the boundary between the yolk and the nucleus, and wander thence to the surface, where they form an epithelial layer round the egg. One would fancy at first that the eminent observer had accidentally confused " test-cells " with the follicle-epithelium, but that he has not done so is evident from his also describing the " test-cells " as formed later on. This discovery is of great interest, both from its upsetting all former theories as to the formation of these cells, which have hitherto always been considered as being formed from the ovary, and also from the singular manner in which Kowalewsky's theory in regard to the formation of the " test-cells " from these cells has been turned upside down, these bodies (*i. e.* the " test-cells ") being formed independently from the yolk (as will be seen hereafter) from which at an earlier period the follicle-cells had also been derived. If M. Fol's observations are correct, it is evident that the term " follicle-cell " is entirely a misnomer, as is also indeed that of " test-cell," both being to a high degree misleading to one who has not studied the history of the appellations.

Within this layer of so-called " follicle-cells " comes the second zone of the egg, consisting of a transparent, apparently homogeneous structure, which, however, when acted on by acetic acid, becomes markedly granular. This is the egg-shell or "chorion" of some authors.

In the interior of the egg-shell and filling it almost completely in the fresh ovum, is the yolk. On the average it measured

.236 mm. and was of a yellowish gray color, due to the coloration of the constituent granules. In the majority of the eggs of this Ascidian I examined, no nuclei were visible either in the fresh egg, or in those that had been subjected to the reaction of acetic acid and glycerine, or osmic acid and carmine. In some, however, a clear spot was noticeable, usually situated eccentrically (in one instance at the periphery of the egg), and measuring .020–.086 mm. One egg presented a rather peculiar abnormality, which I deem worthy of being recorded. The peculiarity consisted in the presence of two distinct nuclei, both situated eccentrically on the same side of the egg and varying somewhat in size, the larger measuring .06 mm. and the smaller .04 mm. I am certain that I did not observe the male and female pronuclei, as the egg under observation had just been removed from the ovary, so that it could not have been impregnated any length of time, if at all, before my observation of it.

These were all the points to be observed in a perfectly fresh ovum, but in one that had been removed from the ovary for a short time, or which had been subjected to the action of various reagents, there was to be seen surrounding the yolk a layer of bodies, which have received the name of "test-cells" from the idea that they were the cells of the developing ovum, from which, eventually, the characteristic test of the adult Ascidian was formed. Kowalewsky [4] in his paper on the development of *Ascidia cunina* states his belief that such is the fate of these cells, which, he also maintains, have their origin from the epithelial cells of the egg-follicle. Later on, however, in his paper on the development of Pyrosoma, [5] he withdraws the statement that the mantle is formed from the "test-cells," but still adheres to the opinion that these are merely follicle-epithelial cells, which have migrated inwards towards the yolk. Before the appearance of his first paper, however, Kupffer, [6] after investigating the subject, came to the conclusion that the "test-cells" formed at the surface of the egg itself, which theory had been independently adopted by Metschnikoff. [7] Before the publication of Kowalewsky's second paper, Hertwig [8] shewed that the "test-cells" take no part in the formation of the mantle, this being formed as a secretion of a homogeneous substance from the epidermis, into which, later on, cells migrate from the epidermis. Hertwig's observations were made on *Phallusia mamillata* and *P. virginea* (?), and have been confirmed by Semper [2] by

observations on *Clavelina vitrea* and *Cynthia depressa.* In the same year that Semper published his observations, a paper by Ussow[9] appeared, in which the old theories first advanced by Kowalewsky are revived and most emphatically insisted upon. He says: "The outer mantle of the Tunicates is developed, not as a secretion product of the epidermal cells of the inner mantle, (Hertwig, Arsenjew,) but by the increase in number and growth of the so-called 'test-cells' (Kupffer, Kowalewski)," and again: "The result of my observations on the formation of the so-called 'test-cells' is in complete accord with that of A. Kowalewski. The yellow bodies are in fact nothing but cells of the Graafian follicle . . . ."

Semper shews that in the several species in which he examined the ova, the "test-cells" were formed in the interior of the egg, and that by the action of reagents, or even by exposure to sea-water, these bodies might be produced in immature eggs. He holds that they are devoid of a nucleus and of a cell-wall, and discarding the term "test-cells," substitutes instead that of "test-drops."

My own observations having been confined to mature or almost mature eggs, I cannot confirm Professor Semper's statement as to the production of these peculiar bodies in immature eggs by means of reagents, but these have the effect of producing them in most cases almost immediately in mature eggs, even the exposure to sea-water for a short time being sufficient for the purpose. Produced in this manner these bodies are small and roundish in shape, and in their interior numerous clear highly-refractive granules are to be seen. I could detect no nucleus either in the fresh or in the stained "drop," and a limiting membrane was also apparently wanting.

As regards their mode of origin I am in accord with the observations of Kupffer,[6] Metschinkoff,[7] etc. When an egg has been removed from the ovary for a few minutes, there appear in the interior of the yolk, numerous clear spots situated nearer the periphery than the centre. In no case did they make their appearance at the centre of the yolk, and though in Figure 3, (Pl. X,) some appear to be very close to it, these in reality are peripheral and appear indistinctly when an optical section of the egg is made and accordingly have been represented. I accordingly conclude that their origin is peripheral as stated by Metschinkoff.[7] They

gradually migrate outwards, until they form a layer at the periphery of the yolk (Pl. X, Fig. 4), and then pass outside of it altogether. The yolk at the same time contracts and leaves a space between its circumference and the egg-membrane, in which the "test-cells" lie, forming at first a layer round the yolk (Pl. X, Fig. 5), but as the contraction of the yolk proceeds, and the space becomes larger, they move away from the surface of the egg and scatter themselves irregularly. (Pl. X, Fig. 6.)

I should imagine that there is in a manner a separation of the egg into two portions; an outer, consisting of protoplasm with comparatively few yolk granules, and an inner, containing most of the yolk granules and a small amount of protoplasm. The outer zone is of no further use in the process of development, and gradually splits up into these "test-drops," their formation commencing at the inner part of the zone and proceeding outwards, until we have numerous "test-drops" and nothing left of the egg but a dense mass of food-granules, closely packed together in the remaining protoplasm, from which the embryo is formed. Metschinkoff [7] describes this separation of the egg into two portions. He says: " In the greenish protoplasm of a young egg of *Ascidia intestinalis*, fine yolk-granules collect round the nucleus; the number of these becomes continually greater, whereby only the peripheral portion of the protoplasm retains its greenish coloration. This layer now separates itself distinctly from the central granular portion and splits up into a great number of round bodies which are the first 'Tunic-cells.'" From this description one would imagine that the author implied that the "Tunic-cells" were formed at the extreme periphery of the egg, which, however, is not the case, for they make their appearance in its interior.

On treating a fresh ovum with a dilute solution of acetic acid (1 or 2 drops of commercial acid to a watch-glassful of water) for about half an hour, its appearance becomes considerably changed. (Pl. X, Fig. 6.) The interglobular protoplasm of the "folliclecells" becomes much more distinct, and, in consequence, the globules themselves become more plainly marked off. The transparent, apparently homogeneous egg-membrane becomes, as mentioned above, distinctly granular. The yolk contracts very much, measuring on the average about .116 mm., half its original size. This contraction leaves a clear space between the yolk and the egg-membrane, which, however, is larger in one-half of its cir-

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cumference than in the other, owing to the eccentric position
assumed by the contracted yolk. In this clear space are numerous
"test-cells," not forming a layer round the yolk, as they usually
do in an egg that has been subjected for a short time only to the
action of acetic acid or sea-water, but scattered irregularly around
the yolk. The "test-cells" measure .008 mm. and present the
appearance described above. In eggs that have been left in acetic
acid for a much longer period (6–20 hours) no further changes
occur, showing that the acid has exerted its full influence on
them.

After exposure to sea-water for six hours, very much the same
appearance is presented as with dilute acetic acid. The "follicle-
cells," however, shew a tendency to separate from the egg-mem-
brane, which, on its part, does not present a granular appearance.
(Pl. X, Fig. 7.)

Upon running some strong picro-carmine under a cover-glass,
below which were some ova in sea-water, very important changes
occurred. At first no "test-cells" were to be seen, but, as the
picro-carmine gradually reached the egg, and the picric acid
exerted its action upon it, it gradually assumed a yellow hue,
while, at the same time, there appeared at its periphery many
small spherical bodies of a round or oval shape, the same size as
the "test-cells," and containing in their interior several highly
refractive granules, which, in fact, render them apparent. No
"test-cells" appear outside the yolk, which retains its original size.
The egg-membrane assumes a pink hue, and, after some time,
becomes distinctly granular. The "follicle-cells" do not stain for
some time and show a tendency to separate from the egg-mem-
brane. (Pl. X, Fig. 8.) The reaction produced by very dilute
picro-carmine is also rather important. After being subjected to
this reagent for about half an hour, the eggs presented an appear-
ance intermediate between that produced by the continued action
of dilute acetic acid and that following the employment of strong
picro-carmine. (Pl. X, Fig. 9.) The yolk contracts to a slight
degree, and "test-cells" make their appearance, filling up the
small space between the partly contracted yolk and the egg-
membrane.

I also employed osmic acid in the following manner. The eggs
were placed in a watch-glass containing sea-water, to which 1 or
2 drops of $\frac{1}{4}$ per cent. osmic acid had previously been added, and

allowed to remain there for from five to ten minutes, when they were removed and stained with Beale's carmine. In most cases no change occurred, the yolk remaining of its original size, and no "test-cells" or clear spots made their appearance in the yolk, with the exception of one instance, in which I did perceive a number of clear spots in the periphery of the yolk.

By these results two questions are suggested: 1st. What are these "test-cells?" 2d. How are the various phenomena caused by the various reagents to be explained? I shall give the second question priority. The explanation that seems to me to be the simplest, and that which bears the stamp of probability most distinctly, is, that these phenomena are caused by the varying effects of the different reagents in producing a contraction of the protoplasm of the yolk. Thus, osmic acid, which "fixes" the protoplasm immediately, allows of little or no contraction, and hence no "test-cells" appear; with picric acid (which evidently is the constituent of the picro-carmine that is active in producing the phenomenon) a slight contraction takes place before the protoplasm becomes "fixed," whereby the "test-cells" are formed, but the contraction is not sufficient to cause them to pass outside the yolk; and, in the last place, with acetic acid and sea-water there is no fixing of the protoplasm, and the contraction goes on to such an extent that the "test-cells" are driven completely outside the yolk. Strong evidence in support of this theory is afforded by the variation in the action of picric acid, according to the strength in which it is used. For, as we have seen, in a dilute solution so much contraction of the yolk is produced, that the "test-cells" do partly pass out.

Accordingly, then, the "test-cells" are formed by a contraction of the protoplasm of the egg, and thus we can readily understand their formation in a developing egg, where the contraction produced by the process of cleavage would be quite sufficient to cause their extrusion from the yolk.

We are now in a position to discuss the question as to the nature of these "test-cells." Semper [2] regards them as merely polar globules, comparing them, in respect to their number, with those of the Mollusca. This theory is, however, untenable, for by the researches of Hertwig on the formation of the polar globules in the eggs of Hæmopis, Nephelis, [10] Asteracanthion, Mytilus, [11] and other forms, we know that the polar globules are formed by a true

cell-division, and are themselves true cells, containing a nucleus, whereas no such process has been observed during the formation of the "test-cells," and I for my part am sure that it does not obtain, and, as Semper himself insists, the "test-cells" are not true cells, but merely "drops." Fol,[3] too, states that in *Phallusia intestinalis* polar globules (two in number) are formed after the disappearance of the original nucleus and after the formation of "test-cells." Accordingly then, there is no morphological homology between the polar globules and the "test-cells." In the eggs of certain forms, however, such as, in the Amphibia, Rana,[10] and in the Pisces, the Trout,[12] after the disappearance of the germinal vesicle, peculiar bodies are extruded from the yolk without any spindle-formation or cell-division, for which Hertwig proposes the name of excreted bodies (Excretkörper) in contradistinction to the polar globules formed by cell-division. These structures have been supposed by the various authors to be the remains of the germinal vesicle, and thus, as far as their mode of formation is concerned, probably do not allow of comparison with the "test-cells," but since they resemble these latter in being bodies whose presence in the egg is not necessary to its further development, and since the cause of their appearance is evidently the same, viz: the contraction of the yolk induced by a stimulus, I think there can be no objection to classifying the "test-cells" with them as Excretkörper.

Wyville Thomson,[13] however, has described bodies as occurring in *Antedon rosaceus* which bear a closer homology to "test-cells" than even these structures. He says: "Consequently on the contraction of the yolk, a number of minute spherical pale yellow oil-globules are apparently pressed out into the space within the Vitelline membrane." These bodies differ from "test-cells" only in the fact that they are oil-globules, whereas "test-cells" are distinctly protoplasmic in their nature, and contain in their interior several oil-globules usually. This distinction, however, is of comparatively little moment, and both in their mode of formation and general appearance these Excretkörper—for so they also may be denominated—are evidently closely related to "test-cells" and perhaps identical with them.

I consider these "test-cells" to be simply masses of albuminous material containing two or three granules of the food-yolk, and presume that they are in reality only portions of the protoplasm

of the egg, which have been forced out by the contraction. If an egg, in which the "test-cells" have passed outside the yolk, be subjected to pressure sufficient to rupture the yolk-membrane, allowing the yolk to come into contact with the "test-cells," and at the same time leaving the egg-shell intact, the "test-cells" commingle completely with the yolk and cannot be distinguished again. The granules to be observed in a "test-cell" have a perfect resemblance, both in shape and appearance, to those remaining in the yolk as food, so that it may be presumed that they are in reality the same, and were originally situated in the yolk, in that portion of the protoplasm which formed the "test-cell," and were extruded with it.

The reason why portions of the yolk, originally of use to the embryo, have become useless and are extruded, must remain undecided until the life-histories of more of the lower types of Ascidians have been fully worked out, but in all probability the explanation is to be sought for in a change in the life of an ancestral form, whereby the development became more rapid and less food-yolk was required, while, at the same time, little or no diminution in the amount of yolk in the egg was produced.

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10. Hertwig. *Beiträge zur Kenntniss der Bildung, Befruchtung und Theilung des thierischen Eies. 2ter Theil. Morph. Jahrb.* Bd. III. 1877.

11. Hertwig. *Beiträge zur Kenntniss der Bildung, Befruchtung und Theilung des thierischen Eies. 3ter Theil. Morph. Jahrb.* Bd. IV. 1878.

12. Oellacher. *Beiträge zur Entwickelungsgeschichte der Knochenfische. Zeit. für wissen. Zool.* Bd. XXII. 1872.

13. Wyville Thomson. *On the Embryogeny of Antedon rosaceus, Linck (Comatula rosacea of Lamarck). Phil. Trans.* 1865.

EXPLANATION OF FIGURES.

Figure 2 is drawn with Hartnack obj. 9, oc. 2; all the rest are drawn with Hartnack obj. 7, oc. 2.

FIGURE 1.—Fresh egg of *Ascidia amphora*.

FIGURE 2.—Follicle-cell.

FIGURE 3.—Egg after short exposure to sea-water.

FIGURE 4.—Egg after longer exposure to sea-water.

FIGURE 5.—Egg after still longer exposure to sea-water.

FIGURE 6.—Egg after exposure for half an hour to the action of dilute acetic acid.

FIGURE 7.—Egg after exposure to sea-water for six hours.

FIGURE 8.—Egg after the action of strong picro-carmine.

FIGURE 9.—Egg after the action of very dilute picro-carmine.

A CONTRIBUTION TO THE STUDY OF THE BACTERIAL ORGANISMS COMMONLY FOUND UPON EXPOSED MUCOUS SURFACES AND IN THE ALIMENTARY CANAL OF HEALTHY INDIVIDUALS.

ILLUSTRATED BY PHOTO-MICROGRAPHS.[1] By GEO. M. STERNBERG, *Surgeon U. S. Army, late "Fellow by Courtesy" of the Johns Hopkins University.* With Plates XI, XII and XIII.

INTRODUCTION.

THE observations recorded in the following paper and the photo-micrographs by which it is illustrated, were made in the Biological Laboratory of Johns Hopkins University, Baltimore, Maryland, during the months of June, July and August, 1881, at which time the writer was acting under the orders of the National Board of Health, and was engaged in special investigations which occupied a considerable portion of his time, and to which this study was necessarily subsidiary.

Microscopists have long been familiar with the fact that a variety of bacteria are constantly found in the alimentary canal of healthy individuals, and that the examination with a sufficiently high power of saliva or fæces never fails to demonstrate the presence of a multitude of these micro-organisms of various forms. Some microscopists to whom this fact is familiar, and whose studies have shown them the widely extended distribution of the bacteria, both within and without the human body, have shown a disposition to ridicule the idea that these minute organisms, so universally present, are capable under any circumstances of playing so important a rôle in the etiology of infectious and epidemic diseases as has been ascribed to them by believers in the "germ-theory." It must be admitted that many extravagant and unfounded claims have been made by over-enthusiastic supporters of this theory, and that a scientific conservatism is very essential to him who would estimate at their true value the facts developed by the numerous re-

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[1] Read at the Cincinnati meeting of the A. A. A. S., Aug. 18th, 1881.

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searches which have been made relating to the bacteria. The literature of the subject is already enormous, and the yearly additions to it seem to grow almost in geometrical progression, showing the rapidly increasing interest in the subject among physicians, sanitarians and men of science generally, due to a more general appreciation of the importance of the questions involved.[1]

It is evident that the time has passed when the spirit of investigation can be arrested by the exhibition under the microscope of the bacteria found in the saliva or fæces of a healthy individual and the magisterial dictum of an "expert microscopist" that these minute organisms are entirely harmless.

That there are many widely distributed forms (species?) which are ordinarily harmless, can not be questioned, but that pathogenic bacteria exist, either as distinct species or as physiological varieties (Pasteur) of common forms, is now definitely proven.

No apology, then, is needed for a study of this nature, the object of which is to place upon record photographic representations of the common bacterial organisms found in the bodies of healthy individuals and some observations relating to their physiological properties and the best method of studying them.

It is evident that a precise knowledge of the morphology and development—life-history—of these common forms is an essential prerequisite to the recognition of unusual forms and to the enlightened study of the possible relation of such forms to any particular disease with which they may be found associated.

I call attention, however, *en passant*, to the fact that recent researches indicate that too much importance has heretofore been attached to morphological distinctions, and that not only may the same organism present distinct morphological peculiarities in different stages of its development, but that during the same stage differences in size, if not in form, may result from conditions relating to the environment—temperature, composition and reaction

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[1] Note.—In the bibliography compiled by Magnin ("The Bacteria," Little, Brown & Co., Boston, 1880) and added to by myself, but which can by no means be considered complete, the references from 1830–40 are seven; from 1840–50, twelve; from 1850–60, seventeen; from 1860–70, sixty-three; from 1870–80, above three hundred and fifty. In the second volume of the "Index Catalogue to Library of the Surgeon-General's Office," just published, four closely printed pages are required for the references relating to "Charbon" alone.

of medium, presence or absence of oxygen, etc. On the other hand, organisms morphologically undistinguishable from each other may possess different physiological properties.

The researches of some of the pioneers in this field of investigation, and especially the discovery by Davaine of a bacillus in the blood of Anthrax and of Obermeier of a spirillum in that of relapsing fever, led many to anticipate that organisms morphologically distinct might eventually be discovered for each specific disease.

This expectation has not been realized, and the germ-theory has been vigorously attacked by conservative opponents who have properly pointed out the morphological identity of *Bacillus anthracis* and *B. subtilis,* and of *Spirochaete Obermeieri* and *S. plicatile* which is not infrequently found in the mouth of healthy individuals. This argument has, however, lost its force, and the common and usually harmless bacteria around us have acquired a new importance since it has been shown by Pasteur,[1] Buchner,[2] Greenfield,[3] Grawitz,[4] and others, that, by special methods of cultivation, pathogenic varieties may be developed from harmless organisms, and that, by certain treatment, deadly bacteria may so far lose their virulence as to produce only a mild, though protective form of disease. In a recent study[5] of "A Fatal Form of Septicæmia in the Rabbit produced by the Sub-Cutaneous Injection of Human Saliva" I have obtained experimental evidence pointing in the same direction.

A brief reference to these facts is all that I can permit myself in the present paper, but I desire to call attention to certain possibilities which remain after the negative demonstration has been made that no organisms are present in the blood of patients suffering from a certain disease—that is, none demonstrable with the highest powers of the microscope as at present perfected. This

[1] "De l'attenuation du virus du choléra des poules." C. R. Ac. des Sc., XCI, p. 373–80.

[2] "Ueber die experimentelle Erzeugung des Milzbrand-Contagiums aus den Heupilzen." München, 1880.

[3] "Further Investigations on Anthrax and Allied Diseases in Man and Animals." Brown Lectures, I–V; London Lancet, 1880, pp. 965–966; 1881, pp. 3–4, 91–94, 163–164.

[4]

[5] See Bulletin National Board of Health, April 30, 1881, and succeeding article in the present number of this Journal.

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negative demonstration by no means proves that the disease in
question is not a germ disease, for the habitat of the parasite may
be elsewhere than in the blood, which may not offer the proper
conditions for its development and from which it may be excluded
by vital or mechanical obstacles.

Bacteria are always present in the alimentary canal of healthy
men and animals, but that they do not find their way into the
blood-stream, or if so, are quickly disposed of, has been amply
proven by the negative results of microscopical examinations and
culture-experiments.

In the form of septicæmia in the rabbit which I have recently
studied, *l. c.*, I have invariably found an abundance of micrococci
in the effused serum in the sub-cutaneous cellular tissue of an ani-
mal recently dead, but these organisms are not always found in
the blood, and my observations indicate that they only invade the
circulating fluid during the last hours of life. Micro-organisms
have been found in many other localities without their presence
being revealed by a microscopical examination of the blood;
e. g., in effused liquids in the pleural and peritoneal cavities, in
pyæmic abscesses, and in various tissues and organs of the body.
I have quite recently found an abundance of minute bacilli in the
substance of the heart of a rabbit, which died as the result of the
sub-cutaneous injection of a contaminated water (unpublished ex-
periment).

The possibility that pathogenic bacteria may become parasitic
upon the bronchial mucous membrane, or in the air-cells of the
lungs, should also be borne in mind. But, when we consider the
extent of the alimentary tract, the variety of substances taken as
food and drink, and the ready access which micro-organisms have
to this human culture-apparatus, kept as it is at a constant tem-
perature and supplied with pabulum suited to their development,
it seems probable that this is the locality where pathogenic organ-
isms may most frequently find the conditions favorable to their
multiplication. This view is supported by many facts connected
with the epidemic prevalence of pestilential diseases, and it is
generally admitted that patients suffering from typhoid fever and
cholera may sow the seeds (germs?) of these diseases in the dis-
charges from their bowels.

It is unnecessary to dwell further upon the possibilities in this
direction which make it important that the bacterial organisms

present in the human body should be studied by modern scientific methods—photography, isolation and cultivation in various media, injection into animals, etc., etc., but I will refer for a moment to another possibility which has occurred to me, which should, I think, receive the attention of chemists and physiologists.

What is the rôle of those micro-organisms which are constantly present in the alimentary canal of men and animals?

The fact that they are parasites does not exclude the possibility of their playing an important physiological rôle in the animal economy.

I am not speaking of accidental or occasional parasites, but of those which have probably been the commensals of man, and of the inferior animals frequented by them, from the earliest times. It can hardly be possible that in the process of evolution the presence of these parasites has had no influence upon the host, or that, to go no further back, in the gradual change from the mode of life and habits of a nomadic savage to that of a civilized man, the change in environment has had no modifying influence upon these micro-organisms, which laboratory experiments show to be so susceptible to changes in temperature and in the composition of the medium in which they are placed.

The question is frequently asked, "If bacteria are such terrible things, how is it possible that we can exist upon the earth surrounded and infested as we are by them?" Certainly there would be an end to all animal life, or rather there would never have been a beginning, if living animals had no greater resisting power to the attacks of these parasites, which by numbers and rapid development make up for their minute size, than has dead animal matter.

On the other hand, but for the power of these little giants to pull to pieces dead animal matter, we should have dead bodies piled up on all sides of us in as perfect a state of preservation as canned lobster or pickled tongue, and there being no return to the soil of the materials composing these bodies, our sequoias and oaks would dwindle to lichens and mosses, and finally all vegetation would disappear and the surface of the earth would be a barren and desolate wilderness, covered only with the inanimate forms of successive generations of plants and animals.

Section 1.

A number of authors[1] have given more or less extended accounts of the micro-organisms found in the human mouth, and their accounts agree so well with each other and with the results of my own observations, that I should hardly think it necessary to record these, but for the fact that I am able to present photographic representations of the organisms described for comparison with the illustrations drawn by other observers.

The special advantages which I claim for this method of illustration are set forth in a paper contained in the last volume (1880) of the Transactions of the American Association for the Advancement of Science.

I would especially call attention to two recent papers, one by Butlin, of England, and the other by Rappin, of France, both of which are illustrated and show careful study.

[1] *Remak.* "Diagnostische und pathologische Untersuchungen." Berlin, 1845, s. 221.

Pfeufer. "Der Mundhöhlen-Katarrh." Henle u. Pfeufer. Ztchft. f. Rat. Med., Bd. 7, 1849, s. 180.

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Koch. "Untersuchungen über Bacteria." Cohn's Beiträge zur Pflanzen, Bd. II, Hft 3, s. 399.

Butlin. "On the Nature of the Fur on the Tongue." Proc. Royal Soc., London, Vol. XXVIII, p. 484.

Rappin. "Des Bactéries de la Bouche." Thése de Paris, No. 144, April, 1881.

Methods of Research.

Collecting.—I have found the following to be the most satis-
factory method of collecting bacteria for examination with high
powers and for photography.

The slightest possible smear of the material to be examined is
allowed to dry upon a thin glass cover, and to secure a sufficiently,
uniform layer, it is usually best to spread it while moist with the
end of a glass slide.

Material is obtained from the mouth by scraping the surface of
the tongue, or of the teeth, with a clean instrument; from the
female vagina by a speculum or digital examination; and from
the mouth of the male uretha by applying a thin glass cover di-
rectly to the moist mucous membrane at the extremity of the canal.

Staining.—A five-cent bottle of aniline violet ink furnishes an
ample supply of staining fluid of the best quality. Two or three
drops of this placed upon the thin cover will very quickly—one
to three minutes—give to the bacterial organisms attached to its
surface a deep violet color. The cover is then to be washed by a
gentle stream of pure water and is ready for immediate examina-
tion, or may be mounted for permanent preservation over a shallow
cell containing a solution of potassium acetate (Koch's method),
carbolic acid water (2–5 per cent.), camphor water, or simply dis-
tilled water.

Photographing.—To make satisfactory photographs of the
smallest bacteria it is necessary to use a staining fluid which will
give stronger photographic contrast, as the violet is transparent
for the actinic rays. I have employed for this purpose aniline
brown (recommended by Koch), or iodine solution (iodine 2–5
grains, potassium iodide q. s. to dissolve, distilled water 100 grains).

A recent writer (Soubbotine[1]) advises the use of osmic acid as a
fixing solution to be used in advance of staining. This is doubt-
less desirable when specimens of blood or thin sections of tissue
containing bacteria are to be examined, as the normal histological
elements are better shown, but the method posesses no special
advantages so far as the demonstration of vegetable organisms is
concerned. It must be remembered that aniline solutions often

[1] Arch, de Phys., 2e série, VIII, p. 479.

contain a granular precipitate which might be mistaken by a novice for deeply stained micrococci.

I cannot here give a detailed account of the technique of the art of photo-micography, but will simply say that there are many difficulties to be overcome, and that the best results can only be obtained by the use of first-class objectives of high power, and by skilful manipulation in the preparation of slides and projection of a well-defined image, supplemented by a sufficient knowledge of the technique of photography to ensure the making of well-timed, well-developed, and properly intensified negatives. For one who has not the services of a practical photographer at his command, the dry-plate process offers many advantages.

Culture-experiments. — A knowledge of the life-histories and physiological properties of the various vegetable parasites which infest the human body can only be obtained by well-devised and carefully conducted culture-experiments. This method of research is still in its infancy, but it has already given valuable results and must doubtless be our main reliance for the advancement of science in this direction. My own experiments have been made chiefly with a view to testing methods and are preliminary to more extended studies which I hope to make in the future.

Culture-cells in which a drop of fluid—aqueous humour, etc.— containing the organisms to be observed, is in contact with a thin glass cover and surrounded by a limited quantity of air, are useful and convenient for certain purposes, especially for the continuous study of successive stages in the development — life-history — of bacterial organisms. But the method of Pasteur—cultivation in gross in sterilized fluids contained in glass flasks—offers decided advantages so far as the isolation, preservation, and cultivation of special forms, and the exclusion of atmospheric germs is concerned; and, also, because the considerable quantity of fluid used gives material for physiological experiments—injections into animals, etc.

The method which I have found most satisfactory, after a considerable number of experiments with various forms of apparatus, is a modification of that of Pasteur which I shall proceed to describe in detail.

The culture-flasks which I employ are shown in Figure 1, Plate XI, supported in small bottles in the position in which they are introduced into the culture-oven.

The larger one, in the centre, is made from a Florence flask, the neck of which has been drawn out into a capillary tube in the flame of a Bunsen burner.   The smaller flasks are of my own manufacture, and are made from glass tubing of about ¼ inch diameter.   Bellows operated by the foot and a flame of considerable size—gas or alcohol—will be required by one who proposes to construct these little flasks for himself, but they could doubtless be obtained at small expense from any thermometer-maker.   A little practice has enabled me to turn out twenty or thirty in an hour, and I have found it much easier to make new tubes than to clean old ones.   I therefore throw them away when they have been once used.

After blowing the bulb the lower end is drawn out in a capillary tube and hermetically sealed in the flame.   In this condition the flask, which is already sterilized by heat, may of course be preserved indefinitely, free from contamination by atmospheric germs.

To introduce a liquid into the flask, heat the bulb slightly, break off the sealed extremity of the tube and plunge it beneath the surface of the liquid.   If the liquid has already been sterilized, temporary exposure to the air while several of the little flasks are being filled is not likely to result in the introduction of atmospheric germs—for any organisms which fall upon the surface of the liquid will be arrested there for a time, unless they are submerged by mechanical means—stirring.

I have found it best, however, not to trust to the sterilization of my culture-liquid previously to its introduction into the flasks, and am in the habit of filling a considerable number of them at one time with filtered chicken-*bouillon*, Cohn's fluid, hay-infusion, or whatever culture-fluid I may desire to use; and, after again hermetically sealing the capillary extremity of the tubes, sterilization of the contents is effected by heat.

This is accomplished by placing the flasks in a bath of oil, melted parafine or concentrated salt-solution, and maintaining them at a temperature of about 105° C. for an hour or more. Occasionally a flask which has an exceptionally thin bulb will explode, and care must be taken by the operator that the hot oil is not thrown into his face by such an accident.   This possibility makes it desirable that a bath should be used having a fixed boiling-point not exceeding 105°, and which consequently does not

require watching. I have found a concentrated salt-solution to fulfil this requirement.

After sterilizing, the flasks are washed to remove the salt-solution from their surface. They are then placed in a culture-oven kept at a temperature of 95–100° Fah. (36–38° C.) for three or four days to test the success of the previous operation—sterilization.

If the liquid contents remain transparent and no mycoderma has formed upon the surface during this time, the flasks may be put aside for future use and can be preserved indefinitely.

The process of sterilization sometimes causes a floculent precipitate to form when albuminous fluids are employed, although they may have been previously boiled and filtered. This might lead to the suspicion that they had broken down, but for the fact that this precipitate is already present when the flasks are introduced into the culture-oven, and no subsequent change takes place.

To inoculate the liquid contained in one of these flasks with organisms from any source, the extremity of the tube is broken off with forceps, the bulb being dependent, and by the application of gentle heat—the heat of the hand is usually sufficient—enough air is forced out to cause a little fluid to be drawn into the tube upon immersing its extremity in the liquid and allowing the air in the bulb to again contract by cooling.

A little experience will enable the operator to inoculate one tube from another, to introduce a minute quantity of blood containing organisms directly from the veins of a living animal, etc., without any danger of contamination by atmospheric germs. No other method with which I am acquainted offers such security as to sterilization of the culture-fluid and exclusion of foreign germs; and a somewhat extended experience in a recent experimental study, "A Fatal Form of Septicæmia," etc., *l. c.*, has convinced me that it has also decided advantages on the score of convenience.

The bottle which supports the inverted flask protects the capillary extremity from dust, and labels are conveniently attached to it. The formation of a mycoderma upon the surface of the fluid is readily recognized, and contained organisms soon settle to the bottom of the tube. Small quantities of fluid are conveniently obtained for microscopical examination by breaking off the end of the tube, forcing out a little of the contents on a clean slide and immediately sealing the extremity again in the flame of a lamp.

Another form of apparatus which I have found very useful is that of Lister, a slight modification of which is shown in Figure 2, Plate XI.

In the apparatus as described by Lister a conical wine-glass contains the culture-liquid, and this is covered by a circular glass plate; the whole being protected from dust by a bell-jar which rests upon a ground-glass plate.

When proper precautions are taken, a sterilized liquid may be preserved in this apparatus for any length of time without undergoing perceptible change. I have used a bell-shaped glass cup having a stem drawn out and sealed in the flame of a Bunsen burner, in preference to a wine-glass, as it is more easily sterilized by heat without danger of breakage. This is supported by a bottle as shown in the figure, and I have commonly dispensed with the use of a glass cover, the use of which is directed by Lister, as I have not found this to be essential to the success of my experiments.

Section II.

Description of Plates, Remarks upon Morphology, etc.

The most conspicuous vegetable organism found in the healthy human mouth, and the one which will usually first attract attention upon microscopical examination with low powers, is the well known *Leptothrix buccalis*, Robin. This I have never failed to find, in greater or less abundance, in material scraped from the surface of the tongue, or in accumulations dislodged from between the teeth. Often it is found in tufts and masses which indicate a vigorous growth, and again it may only occur in the form of short rods sparsely intermingled with the normal histological elements of the saliva, as shown in Figure 2, Plate XIII. But in this case it is probable that a careful search would reveal the presence in the mouth of the microscopic plantations and garden-beds from which these fragments were detached.

As might be expected, those who make frequent use of the tooth-brush leave less soil upon the surface and in the interstices of the teeth for the growth of this and other vegetable parasites. No amount of care, however, will keep the mouth entirely free from them, and the observations of Butlin (*l. c.*) show that the fur upon the tongue, which is rarely entirely absent even in healthy

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individuals, is in great part made up of this and other vegetable parasites.[1]

In Figure 1, Plate XII, several filaments of *Leptothrix* are shown in which evidence of breaking up into joints is seen ; and in Figure 2 of the same plate we have a mass of jointed filaments that seem rather to come under the definition of *Bacillus* than of *Leptothrix*, as given by Magnin in accordance with the classification of Cohn.   This author says: "The *Leptothrix* differ from the Bacilli by their filaments being very long, adherent, very slender, and indistinctly articulated."

These characters seem to me to be very uncertain and unsatisfactory, inasmuch as *Bacillus subtilis* and *B. anthracis*, in one stage of their development, are *very long and slender and indistinctly articulated*, and, on the other hand, we have here a Leptothrix broken up into very distinct joints not distinguishable from those of Bacillus.

The filaments represented in Figure 1 are from a specimen of saliva obtained directly from my own mouth, while those in Figure 2 were developed in a culture-apparatus of special construction (see below) in which a constantly renewed supply of pabulum—chicken-*bouillon*—was passed through a small chamber, freely supplied with air, containing saliva scraped from the surface of my tongue.   Butlin did not succeed in his efforts to cultivate this organism.   He says: "I made many attempts to separate them in order to produce this fungus in a purer form by cultivation, but did not succeed in doing so.   Although this fungus did not develop under artificial conditions in the presence of micrococcus and other fungi, it is highly probable that its development takes place freely upon the surface of the tongue."

It seems probable that my success in the experiment above mentioned is to be attributed to the constantly renewed supply of pabulum and the free access of oxygen, conditions which are certainly present in the mouth, where the surfaces upon which this parasite grows are constantly bathed with saliva and supplied with air.   The author above quoted is of the opinion that the organism in question is identical with *Bacillus subtilis*, and in certain cases he observed "highly refractive spherical bodies which appeared to be spores" in some of the filaments.   I have also observed short

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[1] Butlin found "on 68 healthy tongues—fur on all except one.   On 178 tongues of persons suffering from disease or accident—fur on all except two."

rods containing a single spore at one extremity in specimens of my own saliva examined in New Orleans during the summer of 1880. But at this time similar rods with spores were abundant in certain culture-fluids in my laboratory, and I supposed these to be *Bacilli* accidentally present in my mouth and differing from the common *Leptothrix buccalis*. This is a question, however, which can only be determined by culture-experiments, and I would suggest that the best way to settle it would be to cultivate the *Leptothrix* in an artificial saliva constituted as nearly as possible like normal saliva—but, of course, without the histological elements—and in a culture-apparatus such as was used in my single experiment above referred to. This apparatus is made as follows: A glass receiver *A* having two capillary tubes, one *a* to admit air, and one *b* to permit the gradual escape of the contained culture-fluid, is supported by the bent tube *C*, which is maintained in an upright position by being tied to the cork of a bottle *B*, which answers as a support for the apparatus. Mercury may be placed in this bottle to give it steadiness. The bent tube *C* has a reservoir *e*, which is freely exposed to the air by means of the opening *i*. The organism to be cultivated is introduced into this reservoir. The overflow from *e* is received in the beaker *D*. No attempt is made to exclude atmospheric germs, as the object of the apparatus is to supply, as nearly as possible, the identical conditions found in the human mouth.
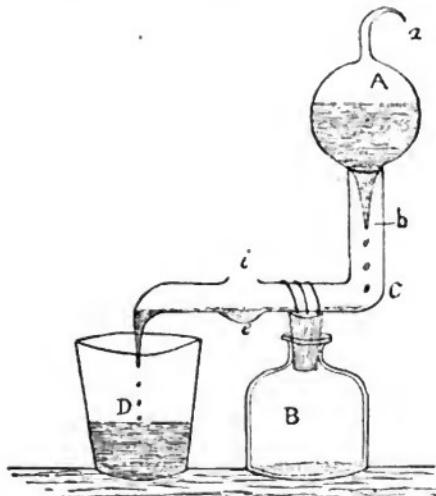
My observations have not been sufficiently extended to justify me in an attempt to describe all of the organisms which are occasionally found in the human mouth, and I shall only refer briefly to the fact that the recorded observations of microscopists indicate that nearly every common bacterial organism known is sometimes found in this situation. This is no more than we should expect, as the germs of these various organisms are widely distributed in the atmosphere and must be deposited upon the moist mucous membrane during inspiration. Their development here will of course depend upon whether the conditions are favorable or otherwise. As these conditions vary within certain limits, we naturally find at different times and in different individuals a variety of organisms present in the buccal secretions differing from those common forms which observations made at distant points [1] show to be constantly present under normal conditions.

Among the varying conditions found in the mouths of individuals considered healthy may be mentioned, a greater or less abundant flow of saliva, a difference in the reaction of this fluid, the presence of decayed teeth, various habits as to food, drink, use of tobacco, etc. The variety of odors to be detected in the breath is sufficient to show that conditions may vary, and it may be that a sufficiently thorough research would result in the establishment of a causal relationship between the presence of certain organisms and the peculiar and offensive odors referred to.

When engaged in the microscopical examination of foul gutter-water and in culture-experiments with various putrifying organic substances, in New Orleans, La., during the autumn of 1880, I not infrequently found nearly every organism in my own mouth which was present in the putrifying liquids under examination, including *Bacterium termo*, *Bacillus subtilis*, *Spirillum undula*, and a variety of minute spherical and rod-like forms difficult to classify except under the general heading of micrococci and bacteria.

A *Spirochæte* not distinguishable from *S. Obermeieri* of relapsing fever has been repeatedly observed by microscopists, but I have not myself met with it.

The *Bacillus* shown in Figure 3, Plate XII, I have reason to believe, from the frequency with which I have found it, is almost

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[1] Robin, Billroth, Butlin, Rappin, and the other authors referred to on page 168.

as commonly present in the healthy human mouth as is the larger and more widely known *Leptothrix*, or *Bacillus*, already described.

This minute organism, which would hardly be recognized without staining and the use of high-power objectives, is also found in normal fæces, if we can trust to the morphological resemblance which will be seen by a reference to Figures 5 and 6, Plate XIII, in which the amplification is the same (1,000 diameters).

Figure 3, Plate XII, is from a culture-experiment in which acid malt-extract (sterilized and tested in culture-oven) was inoculated with a little saliva from my own mouth.

In Figure 4, Plate XII, a fragment of an epithelial cell from the mouth of Dr. K. is shown. The nucleus of the cell is seen at the upper portion of the figure, near this some granules resembling micrococci, and on the margin of the cell a mass of rod-bacteria—probably *B. termo*. Referring again to Plate XIII, Figures 5 and 6, we see that this form also is found in normal fæces. To account for the presence of these organisms in the alimentary canal we have only to suppose that fully developed bacteria, or their unrecognized germs, can withstand the action of the digestive fluids in the stomach and the upper portion of the intestines, and that those, found in the lower bowel, are the direct descendants of those habitually present in the mouth, or of others taken into the stomach with food and drink.

Another organism which I have found quite constantly in specimens of saliva from healthy mouths, although never in any considerable abundance, is shown in Figure 5, Plate XII. This seems to be a *Sarcina* and is, perhaps, identical with *S. ventriculi*, although it presents a somewhat different appearance as to form and grouping from this organism, as shown in a specimen from the stomach in my possession. I have frequently observed little clusters of this sarcina-like organism attached to the surface of epithelial cells in my own saliva and that of others, but to obtain it in abundance I have been obliged to resort to culture-experiments. The figure here given is from a specimen obtained by cultivation in acid malt-extract. This organism, as well as the bacillus shown in Figure 3 of the same plate, multiplies luxuriantly in this fluid when kept at a temperature of 36° C. It may be remarked, *en passant*, that acid malt-extract (a dilute solution) is not unlike the acid fluid ejected from the stomach in cases of obstinate vomiting

attended with the abundant development of *Sarcina ventriculi* in
the stomach.

Figure 6, Plate XII, represents a micrococcus which possesses
an especial interest because of its abundant and constant presence
in the human mouth and because it has been shown to possess
pathogenic properties when injected beneath the skin of a rabbit.
This fact has been brought to light by recent experiments made
independently by Pasteur in France,[1] and by myself in this country,[2]
and since confirmed by Vulpian.[3]

The plate accompanying the paper in which I give an account
of the experimental researches referred to is headed "*Micrococcus
septicus*, Cohn." When this paper was written I thought it prob-
able that the organism represented in my photo-micrographs was
identical with the micrococcus described by Cohn and other ob-
servers under this name. I pointed out, however, that this micro-
coccus is larger than that described by Cohn as *M. septicus*, the
diameter of which is given as 0.5μ, while the organism in question
measures very nearly 1μ. I have since met with a smaller septic
micrococcus which corresponds with Cohn's measurements, and am
now inclined to believe that the micrococcus found in the human
mouth is a distinct species, or at least a well established variety,
differing in size but having nearly the same physiological action
as the *M. septicus* of Cohn.[4]

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[1] Comptes rendus Ac. d. Sc., 1881, XCII, p. 159.

[2] Bulletin National Board of Health, April 30th, 1881.

[3] Bull. de l'Acad. de Méd., March 29th, 1881.

[4] The smaller septic micrococcus above referred to was found under the fol-
lowing circumstances:

EXPERIMENT No. 1, Baltimore, Md., July 9th, 1881.—Injected beneath the
skin of a small rabbit a little material scraped from the mucous membrane of
the intestine of a rabbit just dead. (This rabbit died from an experimental
injection, not yet reported, made for Professor Mallet of the University of Vir-
ginia. It presented upon post-mostem examination evidence of enteritis.)
Result: Found dead at 8 A. M., July 10th. Diffuse cellulitis extending from
point of injection; abundance of minute micrococci in serum from cellular
tissue and in blood from axillary vein; liver, heart, and lungs, normal; spleen
enlarged and softened, but contains no pigment.

EXPERIMENT No. 2, July 10th.—A hypodermic syringe point was dipped in
the blood—from femoral vein—of this rabbit and introduced under the skin of
rabbit No. 2. *Result:* This rabbit was found dead the following morning at
8.30, and a post-mortem examination was made at once with the following
result: Diffuse cellulitis with hemorrhagic extravasations under the skin;
blood from superficial veins full of micrococci; spleen enlarged, softened, dark

In Figure 6, Plate XII, the micrococcus from the mouth is seen as obtained by cultivation (in chicken-*bouillon* inoculated with saliva) in the form of apparatus described on page 169, in which provision is made for a constantly renewed supply of the culture-fluid.

A vigorous development is shown by the grouping in long torula-chains and in zoöglœa masses. In Figure 5, Plate XI, the same organism is shown as found in a culture-flask similar to those shown in Figure 1, Plate XI. In this case the culture-fluid was inoculated with a small quantity of blood taken directly from the vessels of a rabbit just dead as the result of a sub-cutaneous injection of saliva.

The drop of blood used to inoculate the culture-fluid contained the form shown in Figure 6, Plate XI, which differs from that shown in Figure 5 and in Figure 6, of Plate XII, in having a broader areole of transparent material. Identity is proved, however, by the fact that it is directly descended from the last form (Figure 6, Plate XII) and that the first (Figure 5, Plate XI) of which it is the progenitor is morphologically identical with that from which it originated. A reference to Figure 3, Plate VII, in my translation of Magnin's work, "The Bacteria," will show this micrococcus upon an epithelial cell obtained directly from my own mouth. Here also I detect no morphological difference from the form obtained by cultivation in a *bouillon* made from the flesh of a chicken or of a rabbit.

The fact that this micrococcus is the most common organism found in the human mouth and that it has been described by several observers at distant points may seem difficult to reconcile

colored, has rounded edges; liver light colored; lungs congested and present numerous points of hemorrhagic infraction.

EXPERIMENT No. 3, July 11th.—A hypodermic syringe needle was dipped in blood from left auricle of rabbit No. 2 and introduced under the skin of a small rabbit (No. 3). *Result:* This rabbit died at 4.30 P. M , July 13th, but circumstances prevented me from making a careful post-mortem examination, and I have not since had an opportunity to make a more extended study of this form of septicaemia, which, so far as I am able to judge from the experiments made, differs somewhat from the form previously studied by me (*l. c*). The spleen was not so much enlarged and was softer, with rounded edges, corresponding with the spleen of septicaemia as described by Klebs and Tommasi-Crudeli, in their mémoir upon the nature of malarial fever (Studi sulla Natura della Malaria, Roma, 1879). The inflammatory oedema or "diffuse cellulitis" was also less marked.

with the fact, recently developed, that to its presence is due the *exceptional* virulence of the saliva of certain individuals. It accords, however, with the results of recent investigations, which, as already stated in the introduction to this paper, indicate that pathogenic organisms may differ greatly as to their virulent properties as the result of different conditions relating to their environment acting upon successive generations.

My observations lead me to believe that, having a suitable medium, a proper temperature, and a sufficient supply of oxygen, the development or intensification of pathogenic properties depends to a great extent upon an abundant and constantly renewed supply of pabulum. Now this is a condition which differs greatly in the mouths of different individuals. In my own case there is, and has been from my earliest recollection, a very copious secretion of saliva. This, according to my view, accounts for the exceptional virulence which my experiments show it to possess, and is in conformity with the principles of natural selection.

Rapid multiplication is, I infer, an evidence of vigor. Now it is evident that in a natural culture-apparatus like the human mouth the rapid flow of saliva by which contained organisms are constantly washed away will have a tendency to sort out those which develop slowly from those which develop rapidly, and that the former will tend to disappear entirely, while the latter by virtue of their rapid multiplication will survive and the tendency will constantly be to a further development of this property of rapid multiplication. My culture-experiments have shown me that, in fact, this particular microccocus does multiply with great rapidity, and that by virtue of this quality it has the precedence over *Bacterium termo*, the presence of which in any considerable number seems to be fatal to it.

This rapidity of multiplication is shown by the fact that the sub-cutaneous injection of a minute quantity of the material containing it—in the rabbit—results within 24 to 48 hours in the development of an infinite number of micrococci in the effused serum in the cellular tissue, and in the blood of the animal, where they far outnumber the normal corpuscular elements. In my culture-flasks, also, a minute drop of this blood gives rise within a few hours to the development of such a number of micrococci that the fluid contents of the flask are invaded throughout and the pabulum needed for a continued development is exhausted. I

suspect, then, that this is the simple explanation of the phenomenon in question—exceptional virulence—and I am inclined to think that the *modus operandi* of the action of these pathogenic organisms is also to be explained by the possession of this capacity for rapid multiplication.

Nature has placed, or in other words evolution has developed, in the living tissues of animals, a resisting power against the encroachments of bacterial organisms invading and surrounding them, which is sufficient for ordinary emergencies.   But when the vital resistance of the tissues is reduced, on the one hand, by wasting sickness, profuse discharges, etc., or, on the other, the vital activity of the invading parasitic organism is increased, the balance of power rests with the infinitesimal but potent micrococcus.   The rapid multiplication of a micro-organism introduced beneath the skin of an animal is also an advantage in its favor in the way of forestalling the restraining influence of the inflammatory process, which is a provision of nature for building up an impenetrable wall around the invader and thus circumscribing its field of operations.

Experiment has demonstrated that, by some unknown mechanism, the ordinary bacteria of putrefaction and under certain circumstances even pathogenic organisms—*e. g.* after protective inoculations with the micrococcus of chicken-cholera or the bacillus of anthrax—may be introduced directly into the circulation without the production of evil consequences, and that after a short interval microscopical examination does not reveal their presence in the blood.   It is evident that here too a capacity for rapid multiplication and the introduction in the first instance of a considerable number will be circumstances favorable to the parasite and may enable it to get the start of nature's provision for getting rid of it.

NOTE.—It has occurred to me that possibly the white corpuscles may have the office of picking up and digesting bacterial organisms when by any means they find their way into the blood.   The propensity exhibited by the leucocytes for picking up inorganic granules is well known, and that they may be able not only to pick up but to assimilate, and so dispose of, the bacteria which come in their way does not seem to me very improbable in view of the fact that amœbae, which resemble them so closely, feed upon bacteria and similar organisms.

Reference has already been made to Figures 5 and 6, Plate XIII, representing the common bacterial organisms found in

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normal human fæces at the moment of their being discharged from
the rectum.   The photo-micrographs tell the story of the abun-
dance and variety of these organisms, but the present state of
knowledge does not admit of an attempt to determine their phy-
siological rôle in the human economy.   That their constant pres-
ence in the alimentary canal is a fact without import it is difficult
to believe in view of their demonstrated capacity for breaking up
complex organic substances external to the body in the process of
their growth and functional activity.

Figure 4, Plate XIII, shows an epithelial-cell and bacteria from
the orifice of the male urethra.   By gently separating the lips of
the urethra and applying a thin glass cover to the moist mucous
membrane, good specimens are readily obtained of the organisms
commonly found in this locality.

The researches of Lister and my own experiments, shortly to be
detailed, indicate that the healthy human bladder is free from para-
sitic vegetable organisms, and it is probable that those organisms
found at the extremity of the urethral canal, being *aerobic*, do
not extend any considerable distance beyond the orifice.

Lister has shown that urine drawn from the healthy human
bladder with proper precautions may be kept indefinitely without
undergoing change, and Pasteur as long ago as 1862 (Ann. de
Chemie et de Physique, 1862, p. 52.   Comptes rendus Ac. de Sc.,
LVIII, 1864, p. 210) claimed that the alkaline fermentation of
urine is due to the presence of a micro-organism—*Micrococcus
ureæ*, Cohn.   This organism is described by Magnin as follows:[1]
"Oval cells, isolated—diameter 1.5μ (Pasteur), 1.2 to 2μ (Cohn)—
or united by 2, 4, to 8 (*torula*) in a line, straight, curved, zigzag,
or even in cross-form.   In urine of which it transforms the urea
into carbonate of ammonia (Pasteur)."

My photo-micrographs, Figures 3 and 4, Plate XI, show what
I believe to be the organism in question.   The group in Figure 3
answers very nearly to the measurement given, while the arrange-
ment shown in Figure 4 corresponds with that in Cohn's drawing
(Beiträge zur Biologie der Pflanzen, Band I, Heft 2, Taf. III),
although the micrococcus in this figure is smaller.   It is possible
that we have here two different organisms, but I am inclined to
believe that the difference in size is due simply to the fact that

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[1] The Bacteria.   Little, Brown & Co., Boston, 1880.

different stages of development are represented, Figure 4 showing an active pullulating stage and Figure 3 a grouping of the micrococcus in masses after the completion of the transformation of the urea. A difference in the size of individual micrococci will be noticed in Figure 4, and it must be admitted that in photographing these minute organisms with high powers a very slight difference in focal adjustment makes a difference in the apparent size of the organism. Too much stress should not, therefore, be placed upon slight differences of measurement as reported by different observers and obtained by different methods.

I call attention to the fact that this micrococcus has a well defined outline and does not present the appearance of being surrounded by an aureole such as is seen in Figures 5 and 6 of the same plate. This is an additional proof that this aureole is not the result of diffraction, but that it represents a transparent substance enveloping the micrococcus. (See remarks on page 18 of Special Report on "A Fatal Form of Septicæmia," etc. Reprinted from National Board of Health Bulletin, *l. c.*)

The following experiments are reported here as relating to the rôle of this micrococcus, which, notwithstanding the researches of Pasteur, Lister, and others, is not perhaps generally admitted by chemists and physiologists to be *un fait établi.*

Having repeatedly demonstrated the presence of micrococci at the mouth of the male urethra and knowing that Lister's experiments indicate that urine as contained in the healthy bladder is free from bacterial contamination, it occurred to me that in passing urine from a full bladder the first portion of the stream might wash away detached epithelial cells and bacterial organisms, and that the last portion being received in a sterilized flask might give evidence of freedom from these organisms by remaining unchanged. Accordingly I made the following

EXPERIMENT, Baltimore, Md., June 25th, 1881.—Two bell-shaped glass cups were sterilized in the flame of a Bunsen burner and placed under clean bell-jars in the position shown in Figure 2, Plate XI (Lister's Apparatus). I then desired my assistant to pass a small quantity of urine into No. 1 from the first portion of the flow and into No. 2 from the last, removing and replacing the bell-jars as expeditiously as possible. *Result,* June 30th: No. 1 is turbid, has a considerable sedimentary deposit and is decidedly alkaline. No. 2 remains perfectly transparent, has no sedimentary

deposit and is acid. No. 1 contains an abundance of the micrococci shown in Figures 3 and 4, and No. 2 is free from organisms. A single drop taken up from the bottom of No. 1 by means of a pipette was allowed to fall in No. 2. The following day No. 2 was turbid, had an alkaline reaction and contained an abundance of *Micrococcus ureæ.*

This experiment can not be expected to succeed in every instance, as the complete washing away of organisms by the first portion of the stream may not always occur, and it is possible that the previous passage of urine may have washed out the urethra so that the first urine passed will be free from organisms, while the last might be contaminated by the detachment of epithelium in which micrococci were imbedded, as seen in my photographs.

Some such explanation is necessary to account for the result obtained in the following experiment.

EXPERIMENT No. 2, Baltimore, Md., August 1st, 1881.—The above experiment was repeated with the same precautions. *Result,* August 11th: No. 1 remains acid, and transparent in the upper portion, but the lower third is occupied by a loose finely granular precipitate, such as is often seen in fresh urine immediately after cooling. No. 2 has an alkaline reaction, a film of urate of ammonia upon the surface and an abundance of *Micrococcus ureæ* in the copious deposit at the bottom of the cup.

EXPERIMENT No. 3, Baltimore, Md., August 1st, 1881.—Four sterilized cups were prepared as in the preceding experiments and into each was passed a small quantity of urine from my own bladder, after first taking the precaution of disinfecting the extremity of the urethra. This was accomplished by the liberal use of a 3 per cent. solution of carbolic acid, which was applied by means of a pledget of asbestos held by slender forceps. The asbestos was first sterilized by heat and then being dipped in the disinfecting solution was repeatedly and thoroughly applied to the mucous membrane to the depth of half an inch or a little more. This operation produced some pain, and a little soreness upon passing urine was felt for two or three days. *Result:* Five days after (August 6th) the urine in all of the vessels remained transparent. At this date No. 3 was inoculated with organisms from the mouth of the urethra. This was done by twisting around in the orifice of the urethra a small ball of asbestos, previously sterilized by heat, which was then dropped into the cup containing

urine, the bell-jar being removed for an instant only for this purpose. Five days later the contents of the four cups were carefully examined. Nos. 1, 2 and 4 remained transparent, free from sedimentary deposit, and acid. *No. 3 was alkaline and contained an abundance of Micrococcus ureæ.*

A reference to Figure 4, Plate XIII, will show that the organism there seen in considerable abundance is not identical in appearance with *Micrococcus ureæ* as seen in Figures 3 and 4, Plate XI. Direct examination has not given me as satisfactory evidence of the presence of this micrococcus at the extremity of the urethra as have the experiments above detailed. It may be, however, that under different circumstances this organism assumes a different appearance, and that the form shown in Figure 4, Plate XIII, from the surface of a mucous membrane exposed to the air, when submerged in a liquid having the composition of urine undergoes a transformation into the form seen in Figures 3 and 4, Plate XI. This is a question to be settled by carefully conducted culture-experiments.

Figures 1 and 3, Plate XIII, are from the vagina of a healthy female at the termination of the menstrual flow. I shall not here dwell upon the possible import of the presence of micrococci in such numbers in this situation, but from what has already been said it seems evident that gynecologists may well be on their guard to prevent the invasion of wounds in this locality—accidental or made by the surgeon—by these ever-present parasitic organisms, and especially against the development of virulent varieties as the result of profuse and long continued discharges—puerperal, etc.

Figure 7, Plate XI, is introduced for comparison with the other micrococci upon the same plate. The amplification is the same in each—1,000 diameters. This figure is from a specimen obtained by cultivation of micrococci found in gonorrhœal pus, in chicken-*bouillon*. The reader is cautioned against the inference that this micrococcus is the cause of the virulence of the fluid in which it was found. No great weight can be attached to the mere presence of an organism under such circumstances in the absence of culture- and inoculation-experiments to demonstrate its physiological properties. Such experiments I have had no opportunity of making in this case, and the figure is introduced solely for the purpose of showing that distinct morphological differences may be recognized between these micrococci from three different sources, viz: from the human mouth, from urine, and from gonorrhœal pus.

DESCRIPTION OF PLATES.

PLATE XI.

FIGURE 1.—Culture-flasks in position for introduction into culture-oven.

FIGURE 2.—Lister's Apparatus (slightly modified).

FIGURE 3.—*Micrococcus ureæ* × 1,000 diameters by Zeiss's $\frac{1}{18}$ in. hom. ol. im. objective; aniline brown staining.

FIGURE 4.—Same as Figure 3 (see remarks on p. 171).

FIGURE 5.—Micrococcus cultivated in *bouillon* (rabbit flesh) inoculated with blood from septicæmic rabbit, and descended from common micrococcus found in the healthy human mouth, × 1,000 diameters by Zeiss's $\frac{1}{18}$ in. objective.

FIGURE 6.—The same micrococcus as in Figure 5, as it appears in the blood of rabbit killed by the sub-cutaneous injection of human saliva, × 1,000 by Zeiss's $\frac{1}{18}$ in. objective. Iodine staining.

FIGURE 7.—Micrococcus from culture-experiment with gonorrhœal pus × 1,000 diameters by Zeiss's $\frac{1}{18}$ in. objective.

PLATE XII.

FIGURE 1.—*Leptothrix buccalis*, obtained directly from mouth, × 1,000 by Zeiss's $\frac{1}{12}$ in. objective.

FIGURE 2.—*Leptothrix buccalis* from culture-experiment, × 1,000 diameters by Zeiss's $\frac{1}{18}$ in. objective.

FIGURE 3.—*Bacillus* (sp. ?) from culture experiment (saliva in malt-extract), × 1,000 by Zeiss's $\frac{1}{18}$ in. objective.

FIGURE 4.—Portion of epithelial-cell from mouth (Dr. K.) covered with bacteria (*B termo?*), × 1,000 diameters by Zeiss's $\frac{1}{18}$ in. objective.

FIGURE 5.—*Sarcina* (ventriculi?) from saliva-culture in acid malt-extract, × 1,000 diameters by Zeiss's $\frac{1}{18}$ in. objective.

FIGURE 6.—*Micrococcus* from saliva-culture in chicken-*bouillon*, × 1,000 by Zeiss's $\frac{1}{18}$ in. objective.

PLATE XIII.

FIGURE 1.—Epithelium and micrococci from vagina, × 425 diameters by Beck's ½ in. objective.

FIGURE 2.—Epithelium and *Leptothrix buccalis* from mouth, × about 300 diameters by Beck's ½ in. objective.

FIGURE 3.—Epithelial-cell and micrococci from vagina, × 1,000 diameters by Zeiss's $\frac{1}{18}$ in. objective.

FIGURE 4.—Epithelial-cell and bacteria from extremity of male urethra, × 1,000 by Zeiss's $\frac{1}{18}$ in. objective.

FIGURE 6.—Bacterial organisms in normal and recently discharged human fæces, × 1,000 diameters by Zeiss's $\frac{1}{18}$ in. objective.

FIGURE 6.—The same as Figure 5.

A FATAL FORM OF SEPTICÆMIA IN THE RABBIT, PRODUCED BY THE SUB-CUTANEOUS INJECTION OF HUMAN SALIVA.

By GEO. M. STERNBERG, *Surgeon, U. S. A.* With Plate XIV.

In a report made to the National Board of Health in February last, I have given a detailed account of certain experiments, made in the first instance as a check upon experiments relating to the so-called *Bacillus malariæ* of Klebs & Tomassi-Crudelli, which show that my own saliva has remarkable virulent properties when injected into the sub-cutaneous connective tissue of a rabbit. Further experiments, made in the biological laboratory of the Johns Hopkins University, have fully confirmed the results heretofore obtained, and the object of the present report is to place upon record these last experiments, which are of special interest just now because of the announcement by Pasteur, of "*a new disease,*" produced in rabbits by the sub-cutaneous injection of the saliva of an infant which died of hydrophobia in one of the hospitals of Paris. (Comptes Rendus Ac. de Sc., 1881, XCII, p. 159.)

I have demonstrated by repeated experiments—

That my saliva in doses of 1.25 c. c. to 1.75 c. c.[1] injected into the sub-cutaneous connective tissue of a rabbit, infallibly produces death, usually within forty-eight hours.

Query. Do similar results follow the injection of other fluids containing organic matter in suspension or solution?

Answer. One c. c. of my own blood failed to kill a rabbit; 1 c. c. of putrid urine containing *B. termo* in abundance failed to kill a small rabbit; 1 c. c. of liquid fæces and distilled water (1 to 10) failed to kill two rabbits; 1.25 c. c. of *bouillon* undergoing putrefaction and loaded with *B. termo*, failed to kill a rabbit; 1 c. c. of sediment from Baltimore water, consisting of organic

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[1] I have commonly injected an amount varying from 5 to 25 minims, according to the size of the animal, but in small rabbits have had a fatal result in three cases out of five follow the injection of 1 minim diluted with 5 minims of water.

débris and organisms—chiefly *Bacillus subtilis*, *Leptothrix pusilla*, *Protococcus*, and a few diatoms and flagellate monads—failed to kill a rabbit.[1]

On the other hand, injections of a small quantity of surface mud from the gutters of New Orleans during the month of September, 1880, invariably produced fatal results within forty-eight hours. (See unpublished report above referred to.)

Query. Does the saliva of other individuals injected in the same manner produce similar results?

Answer. The saliva of four students, residents of Baltimore (in March), gave negative results; eleven rabbits injected with the saliva of six individuals in Philadelphia (in January) gave eight deaths and three negative results; but in the fatal cases, a less degree of virulence was shown in six cases by a more prolonged period between the date of injection and the date of death. This was three days in one, four days in four, and seven days in one.

Query. Is there any recognizable peculiarity in the saliva which exhibits the greatest degree of virulence?

Answer. In the case of Dr. S., whose saliva shows an exceptional virulence, the teeth are sound, the secretions of the mouth normal in physical properties and reaction, and the general health good. There is, perhaps, an unusual flow of saliva, but no other noticeable peculiarity.

Query. Is there any plausible hypothesis by which this difference in virulence can be explained?

Answer. This question will require for its solution more extended experiments. In the meantime it may be mentioned, as having a possible bearing upon the subject, that Dr. S. has been engaged to a considerable extent, during the past two years, in studies which have brought him in contact with septic material. Dr. F., of Philadelphia, whose saliva killed (after a longer interval) two rabbits, is pathologist to a large hospital, and consequently is constantly brought in contact with septic material. Mr. N. and

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[1] Coze and Feltz found, as the result of numerous experiments, that the blood of healthy persons, and that of persons sick with non-infectious maladies, does not produce fatal results when injected into the sub-cutaneous tissue of rabbits. (Clinical and Exp. Researches upon Infectious Maladies, 8°, Paris, 1872.) Pasteur also has inoculated, without result, the saliva of asphyxiated rabbits and of men dead with common diseases (*l. c.*).

Mr. B., whose saliva killed all the rabbits operated upon (four), are residents of seaport towns in Cuba.[1]

Query. Is death produced in other animals by the sub-cutaneous injection of human saliva, which is virulent for rabbits?

Answer. Injection of 4 c. c. into each of two small dogs produced local abscesses at point of injection, but no other noticeable result.[2] Injection of 0.25 c. c. (each) into five chickens produced no result. Injection of 0.75 c. c. (each) into three guinea-pigs proved fatal to two—one in three and one in seven days. Injection of 0.5 c. c. into five rats resulted fatally to one only.[3]

Query. What is the nature of the fatal malady produced in rabbits by the sub-cutaneous injection of the saliva of certain individuals?

Answer. The course of the disease and the post-mortem appearances indicate that it is a form of *septicæmia.* Immediately after the injection there is a rise of temperature, which in a few hours may reach 2° to 3° centigrade (3.6° to 5.4° Fah.); the temperature subsequently falls, and shortly before death is often several degrees below the normal. There is loss of appetite and marked debility after twenty-four hours, and the animal commonly dies during the second night or early in the morning of the second day after the injection. Death results still more quickly when the blood from a rabbit recently dead is injected. Not infrequently convulsions immediately precede death.

The date and mode of death corresponds with that reported by Pasteur in the memoir referred to. Two rabbits injected with buccal mucus from the mouth of a child recently dead with hydrophobia, December 11th, were found dead December 13th. Other rabbits

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[1] The possibility that this septic condition of the secretions of the mouth may bear some relation to the protection which these Cubans and myself enjoy against yellow fever, which is a disease presenting many points of resemblance to septicæmia, has occurred to me, and without, at present, laying any great stress upon this possibility, I think it worthy of further experimental consideration.

[2] A dog succumbed, however, to an injection of 1 c. c. of serum from the sub-cutaneous cellular tissue of a rabbit recently dead.

[3] The results obtained by me in these experiments correspond with those reported by Pasteur in the paper already referred to, viz: guinea-pig less susceptible than rabbit, complete immunity of the chicken, and susceptibility of the dog to the "new disease" as the result of injections of blood from dead rabbits.

inoculated with the blood and saliva of these died in still less time. Inoculations with fresh blood usually produced death in less than twenty-four hours.

The most marked pathological appearance is a diffuse inflammatory œdema or cellulitis, extending in all directions from the point of injection, but especially to the dependent portions of the body. Occasionally there is a little pus near the puncture, but usually death occurs before the cellulitis reaches the point of producing pus. The sub-cutaneous connective tissue contains a quantity of bloody serum, which possesses virulent properties, and which contains a multitude of micrococci. There is usually more or less inflammatory adhesion of the integument to the subjacent tissues. The liver is sometimes dark colored and gorged with blood, but more frequently is of a lighter color than normal, and contains much fat. The spleen is either normal in appearance or enlarged and dark colored. Changes in this organ are more marked in those cases which are of the longest duration. In certain cases dark colored pigment has been found in the spleen, resembling that which has been supposed to be characteristic of malarial fever. The blood is dark colored, usually fluid, and there is a tendency to agglutination of the red corpuscles.

The blood commonly contains an immense number of micrococci, usually joined in pairs, and having a diameter of about 0.5μ. These are found in blood drawn from superficial veins, from arteries, and from the cavities of the heart immediately after death, and in a few cases their presence has been verified during life ; observations thus far made indicate, however, that it is only during the last hours of life that these parasites multiply in the circulating fluid, and in a certain proportion of the cases a careful search has failed to reveal their presence in *post-mortem* examinations made immediately upon the death of the animal. This organism, however, is invariably found in great abundance in the serum which exudes in considerable quantities from the œdematous connective tissue when an incision is made through the integument over any point involved in the inflammatory œdema extending from the original puncture.

A perusal of the paper of Pasteur, already referred to, has induced me to pay especial attention in three recent *post-mortems* to some points to which this author refers, which I had not noticed in previous

examinations, viz: to the condition of the trachea, the lungs, and the lymphatic glands in the groins and axillæ.

Pasteur says, "The cellular tissue is almost always emphysematous." (This has not been observed to be the case, except to a slight extent in one instance in the rabbits operated upon by me.) "The lungs are frequently filled with the *noyaux* of pulmonary apoplexy." (I have found this to be the case in one out of three rabbits examined since my attention has been directed to this point.) "A character more constant than the last (not more constant, however, than that which relates to the volume and color of the *ganglions*), is the state of the trachea, which is almost invariably red, congested, with little hemorrhages from the smallest vessels." (I have found a marked congestion of the vessels of the trachea in the three cases in which I have examined it, and in one case the lymphatic glands of the axillæ were enlarged and congested.)

Query. What constituent of the saliva injected produces the fatal malady in question?

Answer. The following facts demonstrate that *the phenomena detailed result from the presence of a living organism found in the saliva—a microccocus—which multiplies abundantly in the subcutaneous connective tissue, and also in the blood shortly before or after death.*

(a) *The poison is particulate.* This is proved by numerous filtration experiments. *Example*: March 15, 11 A. M. Injected 1 c. c. of filtered saliva (filtered through thin stratum of plaster of Paris, by means of Sprengel's pump) into left flank of rabbit weighing 1 pound, and at the same time one-fourth the quantity of unfiltered saliva into a rabbit of the same size. No harm resulted to the first rabbit, while the second died the following day, at 5.30 P. M.

(b) *The virulence of the saliva is destroyed by boiling.*

(c) *The saliva loses its virulence when kept for twenty-four hours in a culture-chamber, at a temperature of 37° centigrade.*

The presence of *B. termo* and an odor of putrefaction in saliva kept for twenty-four hours in a culture-chamber shows that changes are occurring which have heretofore been recognized as destructive of the septic poison (organism), *e. g.*, the virulence of the poison which produces dangerous dissection wounds is lost when putrefactive changes set in.

(d) *The addition of one part of a 10 per cent. solution of carbolic acid to two parts of saliva destroys its virulence.*

(e) *The effused serum from the sub-cutaneous connective tissue of
a rabbit recently dead, produces death attended with the same phe-
nomena as resulted from the injection of the saliva in the first instance.*
But this does not contain epithelial cells or salivary corpuscles,
and we are, therefore, justified in excluding these as possible agents
in the production of the results indicated. Moreover, these are
present at all times in the saliva of all individuals, while viru-
lence, at least such an intense degree of virulence, is an exceptional
property of human saliva.

(f) *This serum loses its virulence by filtration.*

Unfiltered serum from a recently dead rabbit has invariably proved
fatal in smaller quantity and in less time than is required by the saliva
in the first instance, showing an increase of virulence as the result of
successive cultivation of the organism in the body of a susceptible
animal. This corresponds with the results obtained by Davaine, Koch,
Pasteur and others. I have not attempted to ascertain the minimum
quantity which will produce death. Davaine says : "A rabbit may be
killed by the $\frac{1}{1000}$ part of a drop of septic blood." (Bull. de l'Acad.
de Med., 2 s., T. VIII, p. 121.) In my filtration experiments I in-
jected, however, quantities far in excess of the amount required to
produce speedy death if unfiltered serum had been employed.

Example : March 14. Injected 2 c. c. of filtered serum (from sub-
cutaneous connective tissue of rabbit recently dead) diluted with dis-
tilled water (1 to 20) without result, while one-quarter the quantity
(0.5 c. c.) of the same dilution *unfiltered*, injected at the same time
into another rabbit, produced death in twenty-four hours.

(g) *The micrococcus present in the serum from the connective tissue
of a rabbit which has succumbed to a sub-cutaneous injection of
saliva, may be cultivated in bouillon made from the flesh of a healthy
rabbit, or in blood serum obtained from a healthy dog, and these
fluids thereby acquire a virulence which they did not have before.*

My first efforts to cultivate the micrococcus in urine, in gelatine solu-
tion, and in *bouillon* made from the flesh of a dog, all proved inef-
fectual, and these fluids after inoculation with blood or serum from the
connective tissue, showed a temporary virulence only, which was doubt-
less due to the presence of the micrococci introduced, which preserved
their vitality for a certain time, although the conditions were not
favorable for their increase. After a few days the first culture lost its
virulence and successive inoculations gave negative results, both as to

the presence of the micrococcus and as to noxious properties when injected into rabbits.

(*h*) *Successive cultures in which but a small drop is taken each time to inoculate a fresh quantity of bouillon exclude the white and red blood corpuscles* (filtration-experiments have already shown the poison to be particulate) *as possible agents in the production of this virulence, and prove conclusively that the veritable cause is the presence of a micrococcus,* found first in the saliva, then in the serum from the connective tissue, and (usually) in the blood of the animal killed by the injection of saliva, and finally in each successive culture-fluid inoculated (in the first instance) with a small quantity of this serum or blood.

Within a few hours after inoculating sterilized *bouillon* made from the flesh of a rabbit (first tested for several days in a culture-oven at a temperature of 37° Cent.) with blood, or serum from sub-cutaneous connective tissue of a rabbit recently dead, the fluid—previously transparent—becomes opalescent, and upon microscopical examination is found to contain innumerable micrococci, solitary, in pairs, and· in torula chains. The same result follows upon inoculating a second portion with a minute drop of the first, and so on. The continued virulence of these successive cultures I have amply proved.

Example: April 13. Injected 1 c. c. of *bouillon*-culture, No. 6 (six successive inoculations, the first with serum from sub-cutaneous connective tisssue of rabbit), into left flank of a large rabbit. *Result:* The animal was found dead on the morning of the 16th, and presented the usual appearances upon *post-mortem* examination. Its blood and the effused serum in sub-cutaneous connective tissue contained, as usual, an immense number of micrococci, like those already described.

Query. Does the micrococcus found under the circumstances detailed differ from the *Micrococcus septicus* of Cohn, and is it identical with the organism described by Pasteur, as present in the blood of rabbits killed by the sub-cutaneous injection of the saliva of an infant dead from hydrophobia (*l. c.*)?

Answer. Cohn describes the *M. septicus,* as follows :

" Little rounded cells of 0.5 μ, motionless and crowded in masses, or united in chaplets in the secretion of wounds in cases of septicæmia (Klebs), in *zooglœa* in callous ulcers, in isolated cells, united *in pairs* or in chaplets in the serum of *epidemic puerperal fever* (Waldyer), in all the tissues, vessels, etc., in cases of pyæmia and septicæmia." (The Bacteria, Magnin : Little, Brown & Co., Boston, 1880, p. 76.)

Pasteur gives the following description of the micrococcus found by him in the fatal disease described by him as new, and which he evidently does not consider identical with septicæmia, a disease which he had previously studied experimentally. It should be noticed, however, that Pasteur recognizes several forms of septi-cæmia. Thus he says:

" And now we see why septicæmia has so often been confounded with charbon; their causes are of the same order; it is a vibrio which causes septicæmia and a bacillus which produces charbon. * * * Septicæmia and putrefaction in a living being are not the same thing. *There are as many different septicæmias as there are different vibrios.* * * * In septicæmia the vibrios do not appear in the blood until the last thing, but in this liquid one of them takes a peculiar aspect, often longer than the diameter of the field of the microscope, and so transparent that it easily escapes observation; when, however, it is once perceived it is easily found again, flexible, climbing and removing the blood globules as a serpent moves the grass in the bushes," etc. (Charbon and Septicæmia, C. R. Ac. des Sc., LXXXV, 101–115.)

This septic vibrio of Pasteur I found in the blood of rabbits, victims of my experiments, in New Orleans during the past summer (Report to National Board of Health, not yet published), but have not since met with it; perhaps because it develops *post mortem* and requires the hot weather of summer for its development. Whether it is an independent organism or is developed under special conditions from the *Micrococcus septicus*, being an advanced phase in the development of this organism corresponding with the spore-producing filaments which have been shown to constitute one phase in the life-history of *Bacillus anthracis*, Koch, and of *Bacterium termo*, Ewart, is an interesting question for further research. The vivid language of Pasteur describes it well, and the wonderful vigor with which this extremely slender and almost transparent organism thrusts aside the blood corpuscles in its impetuous serpentine movements cannot fail to astonish the observer. The micrococcus of Pasteur's "new disease" is, on the contrary, quite motionless, and is described as follows:

"This organism is sometimes so small that it may escape a superficial observation. Its form does not differ from that of many other micro-scopic beings. It is an extremely short rod a little compressed towards the middle, resembling a figure 8, and of which the diameter of each

half often does not exceed a half a thousandth of a millimeter [= 0.5 μ and corresponding with the diameter given by Cohn for the *Micrococcus septicus*, also with the micrococcus observed by myself in the form of septicæmia described in this report]. Each of these little particles is surrounded at a certain focus with a sort of aureole which corresponds, perhaps, to a material substance." (NOTE.—The possibility that this appearance is due to diffraction is considered, but Pasteur inclines to the opinion that in the case in question it is due to a mucous substance which surrounds the organism.)

The foregoing descriptions answer as well for the micrococcus observed by me as if they had been written especially for it, and it is unnecessary for me to say more at present in relation to the morphology of this organism, which apparently is identical with that of the *Micrococcus septicus* of Cohn, and with the organism found by Pasteur in the "new disease" described by him. Does it then follow that the organisms are identical, and that the phenomena related by Pasteur, as resulting from the sub-cutaneous injection of saliva from an infant dead of hydrophobia, and by myself, from saliva of a healthy adult, represent the same disease? By no means.

The argument, that because a certain bacillus, or spirillum, or micrococcus, is morphologically identical with another, which is proved to be harmless as to its effects upon an animal organism, consequently *it* must be harmless, has no support from analogy or experiment. The argument is: Bacteria are found everywhere, we eat them, we drink them, we draw their germs into our lungs at each inspiration and without apparent injury. They are evidently harmless. Your spirillum of relapsing fever does not differ (the morphological resemblance is admitted) from a harmless spirillum frequently found in the human mouth; your *Bacillus anthracis* does not differ from *Bacillus subtilis*, etc. The answer is plain. The fact that there are harmless bacteria does not disprove the possibility of pathogenic bacteria; the fact that two things look alike does not prove that they are alike; experiment proves conclusively that the phenomena of anthrax are due to the presence and multiplication in the body of the affected animal of the *Bacillus anthracis*, and that in the fatal form of septicæmia described in this report, the efficient cause of the morbid phenomena, and of death, is the minute micrococcus described.

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Doubtless, harmless micrococci abound. Pasteur finds no difference, morphologically, between the organism which produces the "new disease" described by him and that which produces the *cholera des poules.* He says : " By the form which it has in the blood the organism resembles the microbe of chicken cholera, but it differs completely in its functions. We may inoculate fowls with it without their experiencing the slightest ill effect." (The same is true of the organism producing the form of septicæmia described in this paper.)

"In the form of chaplets it resembles greatly many other organisms which I have often observed," etc.

It will have been noticed from the account already given that the fatal disease in rabbits observed by me and resulting from the sub-cutaneous injection of my own saliva resembles in many particulars the disease described by Pasteur as new, resulting from the sub-cutaneous injection of the saliva of a child dead with hydrophobia. Another point of resemblance is the fact that the saliva of one of my rabbits, recently dead, has the same virulence as the blood and serum from connective tissue. A serous liquid, which in some instances escapes from the bowels shortly before or after death, also contains the micrococcus in abundance and possesses like virulence. All of these points of resemblance form a strong probability in favor of the identity of the two diseases, but I am not prepared to pronounce a positive opinion upon this point, especially since Pasteur, who had previously given much attention to the study of septicæmia, pronounces the disease observed by him to be new, while I see no reason, at present, for supposing that the disease observed by me differs essentially from the experimental septicæmia produced by Davaine, Koch and other investigators, who, however, obtained their first supply of septic organisms from a different source.

In the light of what we already know, it seems very probable that puerperal fever, hospital gangrene, and the various forms of septicæmia known to physicians and surgeons result from the development of pathogenic varieties of harmless and widely-distributed species of micrococci, as the result of especially favorable surroundings; such as are found in the lochial discharges of a puerperal woman or in the secretions from the surface of wounds in a crowded and ill-ventilated hospital ward.

Just as differences in resisting power to experimental septi-cæmia are exhibited by different species of animals, so doubtless individual differences exist in man, especially as the result of lowered vitality; and this want of resisting power, from whatever cause resulting, must be counted as one of the conditions favorable to the development and propagation of a pathogenic bacterium. Thus we find that in experimental septicæmia the micrococcus does not invade the blood until the vital powers are at a low ebb, and death is near at hand.[1]

In the dog the vital resistance is competent to withstand the assaults of a micrococcus—injected sub-cutaneously—having the potency of those found in my saliva, and the result of such an injection is simply a circumscribed abscess. But the increased power (which is perhaps simply a more vigorous and rapid devel-opment) gained by cultivation in the body of the rabbit, enables these organisms to overcome the resistance of the dog, and a diffuse cellulitis results of a faïal character.

The fact, observed by myself, that during the summer months the mud in the gutters of New Orleans possesses an extraordinary degree of virulence[2] shows that pathogenic varieties of bacteria are not alone bred in the bodies of living animals. The more I study this subject the more probable it seems to me that in this direction lies the explanation of many problems which have puzzled epidemiologists, and that the sanitarians are right in fighting against filth as a prime factor in the production of epi-demics—a factor of which the *rôle* is easily understood, if this view is correct.

The presence of septic organisms, possessing different degrees of virulence, depending upon the abundance and kind of pabulum furnished them and upon meteorological conditions more or less favorable, constitutes, in my opinion, the *epidemic constitution of the atmosphere,* which wise men were wont to speak of not many years ago as a cloak for ignorance. It must be remembered that the

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[1] By virtue of some property or mechanism at present unknown, blood, which external to the body is a favorable medium for the development of many spe-cies of bacteria, resists their entrance or gets rid of them when they effect an entrance, *e. g.,* by injection, so long as it is circulating in the vessels of a healthy individual.

[2] There is no reason to suppose that this is peculiar to New Orleans, but I have not yet had the opportunity to extend my experiments to other places.

gutter mud of to-day, with its deadly septic organisms, is the dust of to-morrow, which in respiration is deposited upón the mucous membrane of the respiratory passages of those who breathe the air loaded with it. Whether the peculiar poison of each specific disease is of the same nature or not—a question which can only be settled by extended experimental investigations in the future—it is altogether probable that this factor often gives a malignant character to epidemics of diseases which, uncomplicated, are of a comparatively trivial nature.

PART SECOND.—MORPHOLOGY.

Since writing the report published in *"The Bulletin,"* of April 30th, my attention has been called to the fact that M. Vulpian has arrived at similar results (Bull. de l'Acad. de Med., March 29, 1881); and I infer that Pasteur has somewhat changed his opinion as to the nature of the "new disease," described by him in his communication to the French Academy (made January 26th), from the following remark of Chauveau, which I find in his recent address, as President of the French Association for Advancement of Science. (Revue Scientifique, April 16th, 1881.) He says:

"For a moment we hoped that Pasteur had determined thus" (by artificial cultivation) "the virus of hydrophobia, *but he tells us himself that he has only cultivated a new septic agent.*"

There seems, therefore, to be no longer any reasonable doubt of the identity of the "new disease," described by Pasteur, and the fatal form of septicæmia in the rabbit produced by the sub-cutaneous injection of human saliva, which I first observed in New Orleans, in September, 1880, and which I have since studied, experimentally, in Philadelphia (in the Medical Department of the University of Pennsylvania, in January), and in Baltimore (in the Biological Laboratory of Johns Hopkins University).

Having proved, experimentally, that the presence and multiplication of a micrococcus is the essential feature in the etiology of this disease, a further study of the morphology of this minute organism becomes of interest.

In Plate XIV, Figure 1 represents the organism as found in the blood of a rabbit recently dead; Figures 2 and 3, the same from a culture-solution (*bouillon* made from the flesh of a rabbit);

Figure 4, the same as found in human saliva, while Figure 5 represents a micrococcus from another source, introduced for comparison. The amplification in each case is 1,000 diameters, and the photo-micrographs, which have been accurately reproduced by the heliotype process, were all made with the same objective (Zeiss $\frac{1}{18}$ hom. im.) and at the same distance, with the exception of Figure 4, which was made at a different time with Zeiss $\frac{1}{9}$ in. and a longer distance.

The first thing which strikes an observer upon an inspection of this Plate will, doubtless, be the fact that the organism presents very marked morphological differences in the figures given, and the question will at once arise as to a possible mistake in identity.

So far as the form represented in Figure 4 is concerned, it must be admitted that there is no positive evidence that this is really the septic micrococcus as found in human saliva, which is the parent of the form developed in the blood of the rabbit, and represented in Figure 1. What is positive and invariable, so far as my experiments go, is that the injection of my saliva into the sub-cutaneous connective tissue of a rabbit is followed by the appearance in the effused serum, and subsequently in the blood (usually) of the micrococcus seen in Figure 1, and that this is a septic organism. As the saliva contains a variety of bacteria, including rod and spiral forms, as well as micrococci, it may be supposed that the form developed in the blood of the rabbit as the result of the sub-cutaneous injection of this fluid is descended from any one of these forms. But while there is no positive proof to this effect, the abundant presence of the micrococcus represented in Figure 4, and its morphological resemblance to the form shown in Figure 1, makes it seem highly probable that this, rather than one of the other forms referred to, is the parent form in this case.

It will be noticed that in Figure 4 the micrococcus is more abundant over the epithelial cell than around it, and, indeed, the cell seems to be invaded by the organism as if it were parasitic upon it. This is a very common appearance, and in many cases the epithelial cells are seen to be invaded to a greater extent even than in the example which has served for this photo-micrograph.

It must be remembered that micrococci, morphologically resembling these, are commonly found in saliva which does not possess marked virulent properties as well as in that which does, and if the organism is specifically the same in both cases, we must admit

the existence of varieties possessing physiological peculiarities, although morphologically identical.

It may be well to say here that the sharply defined photographic image of these minute organisms which is seen in the figures, can only be obtained by staining processes and by the use of first-class objectives. A failure to demonstrate the presence of this micrococcus with a ½ or ⅛ inch objective without the use of a suitable staining fluid, cannot be accepted as proof of its absence.

In these photographs the staining was affected with iodine solution, as the yellow or brownish color which this gives is well adapted for giving strong photographic contrast. When there is a distinct cell wall, as in the larger bacteria, leptothrix, etc., a still better effect can be obtained by first covering the organisms (dried upon a thin cover) with strong sulphuric acid for a very short time (one or two minutes), and after washing this off by a gentle stream of water, immersing the cover in a weak solution of iodine (iodine, grs. iii, potassic iodide, grs. v, distilled water, grs. 200) for a few minutes.

For ordinary microscopical examination, I have found no staining fluid equal to a solution of aniline violet, first recommended by Koch.

The most striking morphological difference between the micrococcus as shown in Figures 2, 3 and 4, and in Figure 1, is the aureole which surrounds the well-defined dark central portion in the latter figure.

Pasteur says of this appearance: "This organism is sometimes so small that it may escape a superficial observation. . . . It is an extremely short rod, a little compressed towards the middle, resembling a figure 8. . . . *Each of these little particles is surrounded at a certain focus with a sort of aureole which corresponds perhaps to a material substance.*"

Pasteur's inference that this aureole represents a material substance, and is not simply the result of diffraction, is fully sustained by my observations and my photographs. The slighter aureole seen in Figures 2 and 3 is probably a result of diffraction; but the use of aniline violet as a staining fluid promptly demonstrates that in Figure 1 we have to do with a material substance. The refractive index of this substance must be very nearly that of blood serum, for it is with great difficulty that this aureole can be distinguished without the aid of staining material. It may be

seen by the practiced eye with a good immersion lens, but, as already mentioned, even the darker central portion, which alone is seen at first, may easily escape observation, and a false impression is obtained as regards the real size of the organism. When, however, a small drop of blood, dried upon a thin glass cover, is immersed for a minute or two in a solution of aniline violet, and then washed and examined with, even, a good ⅛ inch objective, the observer will be astonished to find a multitude of organisms, solitary, in pairs, and in chains, having a diameter of more than 1 *μ*, and mostly possessing an oval or elongated form, which might lead to the inference that they should be referred to the genus *Bacterium*, Duj., rather than to *Micrococcus*, Cohn.

The reason of this apparent change in dimensions as the result of staining, is that the substance which constitutes the almost invisible aureole is deeply stained by the aniline, and the central portion, which was before seen because of its highly refractive index, is now lost to view in the uniform and deep violet color which the whole organism possesses.

A careful study of Figure 1 will show that the inference which might be drawn from the examination of a specimen stained with aniline violet as to the oval or rod form of the organism is not a correct one. It will be seen that a certain number of spherical (micrococcus) organisms are seen in the field, and that the oval and elongated forms evidently represent successive stages in the process of fission, which is seen on the point of completion in the figure eight (8) form, in which two spheres are coupled together and enveloped in a transparent matrix. It may be necessary to explain that the large, dark colored, and ill-defined objects in the field, are blood corpuscles changed in appearance by the action of the iodine solution used for staining the micrococcus. (Fig. 1.)

When a culture-tube containing *bouillon* made from the flesh of the rabbit is inoculated with a minute quantity of blood taken from a rabbit recently dead and containing the organism shown in Figure 1, and placed in an oven at a temperature of 37° Cent., there is a rapid multiplication of the micrococcus, which, it is proved experimentally, retains its virulent properties. While in process of active multiplication the organism also retains, at least to some extent, its characteristic form as shown in Figure 1, and presents the appearance of being surrounded by an aureole as

already described. But in a limited amount of the culture-fluid
the process of multiplication by fission soon ceases.

Observations thus far made indicate that from six to twenty-four
hours' time is sufficient to exhaust the capacity of the culture-fluid for
sustaining the development of the organism.

When the liquid is examined during the first few hours after
inoculation, it is seen to be slightly opalescent, and upon micro-
scopical examination is found to contain, distributed through it,
an abundance of micrococci, solitary, in pairs, and in short chains.

At a later period (48 hours) the micrococcus will be found
chiefly at the bottom of the fluid in groups or zoöglœa masses as
seen in Figures 2 and 3, and without the aureole of transparent
material which characterizes it, especially in the blood of the rab-
bit, during its active multiplication. There can be no question
that we have here the same organism for this culture-liquid in-
jected into the sub-cutaneous connective tissue of a rabbit produces
fatal septicæmia, and the blood of the victim swarms with the
form shown in Figure 1.

If a culture-liquid in which the micrococcus has been present
in abundance be examined at a later date (one or two weeks), the
organism will be no longer found, at least in a recognizable form;
and, so far as my experiments go, the culture-liquid no longer
exhibits any virulence when injected beneath the skin of a rabbit.

My experiments thus far indicate that no germs are formed in
the blood or in culture-tubes, which may be preserved for an indefi-
nite time, and then employed for starting a new series of culture-ex-
periments, as in the case of *Bacillus anthracis*, etc. I design making
further experiments in this direction, however, and from what is known
of the life-histories of allied organisms, we have reason to expect that
permanent spores may be obtained capable of preserving their vitality
indefinitely, when the conditions of their development have been more
fully studied.

From what has been already said, and from a critical study of
my photo-micrographs, it will be seen that the measurement given
by Cohn for *Micrococcus septicus*, by Pasteur for the organism
described by him, and by myself in my first paper, viz: 0.5μ, is
too small for the organism represented in my photo-micrographs.
The amplification in these is exactly 1,000 diameters, if the mi-

crometer plate in my possession is accurate (Powell & Leland's). But, according to my measurements, the micrococcus as shown in Figures 2 and 3 is but little less than $1\,\mu$ in diameter, while the organism as shown in Figure 1 is of nearly twice this diameter, when the aureole is included in the measurement. The most reliable measurement is, perhaps, to be obtained from the group shown in Figure 3, in which the micrococci may be supposed to touch each other. By measuring two or three lying in juxtaposition in a right line, we reduce the probable error which in a single one results from the somewhat uncertain outline of the organism as shown in a photo-micrograph. Adopting this method, I obtain an average diameter of $\frac{1}{37000}$ of an inch from Figure 3, and $\frac{1}{14000}$ of an inch from Figure 1 (including the aureole). It must be remembered that slight differences are likely to be deceptive, as it is impossible to obtain exactly the same focus in every instance, and the apparent size is influenced to some extent by the particular focus at which the picture is taken, and possibly also by the staining material employed.

Figure 5 is introduced to show that there are micrococci and *micrococci.* The species (?) here represented was obtained in the first instance from gonorrhœal pus. A little of this pus, obtained from a case of two weeks' duration, showed upon microscopical examination, in a few of the pus corpuscles, an invasion by micrococci, while the majority of the corpuscles, as well as the liquid in which they were suspended, were free from organisms. A culture-tube containing sterilized *bouillon* (from rabbit) was inoculated with a little of this pus, and an abundant development of micrococcus resulted. A second tube was inoculated from the first, and a third from the second. The organism was found in abundance in all of these solutions (kept in a culture-oven at 37° Cent.), unchanged in appearance and unmixed with any other forms of bacteria. One cubic centimetre of the liquid from culture No. 3 was injected under the skin of a small rabbit with an entirely negative result. It is evident, then, that physiologically this micrococcus differs from the deadly septic micrococcus which we have been studying. It also presents slight morphological differences. It is a little smaller and is more easily seen than the *M. septicus* when examined without previous staining. This is because it has a little color (?), or refracts light differently from the latter, and not being surrounded by an aureole of transparent material, it

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presents a more definite outline. A slight aureole due to diffraction will, however, be seen upon closely inspecting the photograph.

The question will naturally be asked as to the possible relation of this organism to the peculiar virulence of gonorrhœal pus. I have not yet found time to study this question experimentally, but think it quite probable that this organism will be found to be identical with the micrococcus found in pus from other sources, *e. g.*, open wounds, inflamed mucous membranes, etc. Whether this common and widely distributed micrococcus is capable under special conditions of cultivation of developing into various pathogenic micrococci; whether it is a distinct species from our septic micrococcus, or whether the latter is a pathogenic variety developed from it, are questions which can only be settled by extended and painstaking experimental investigations.

EXPERIMENTS WITH DISINFECTANTS. By GEO. M. STERNBERG, *Surgeon, U. S. A.*

In experiments previously reported (National Board of Health Bulletin, Vol. 1, Nos. 29, 30, 37 and 47), the comparative value of certain well-known and commonly-used volatile and gaseous disinfectants was tested. In these experiments vaccine virus was the substance exposed to the action of disinfectants, and the test of disinfection was insertion of the disinfected virus into the arm of an unvaccinated child, virus from the same source not disinfected being inserted at the same time at a different point. A positive result from the non-disinfected virus and a negative result from that exposed to the disinfecting agent was taken as proof of the potency of this agent.

As an additional test some experiments were made upon the bacteria contained in putrid urine, the test of disinfection being the failure to multiply in sterilized urine after exposure to the action of a disinfectant. (See Bulletin Nos. 37 and 47, Vol. 1.)

The general results of these experiments may be stated as follows:

Chlorine.—In experiments upon vaccine virus, dried upon ivory points, an exposure for six hours in an atmosphere containing 5 volumes to 1,000 of air ($\frac{1}{2}$ per cent.) was found to destroy the potency of the virus. A still smaller quantity ($\frac{1}{4}$ per cent.) was found to destroy the vitality of bacteria dried upon a piece of filtering paper, and it is possible that further experiments would have demonstrated the efficiency of this agent in still smaller quantities.

Nitrous acid gas (generated by pouring nitric acid on copper filings and collected over mercury) destroyed the potency of vaccine virus in the proportion of 1 per cent. (1 volume to 100 of air); time of exposure six hours. The experiments upon bacteria showed this agent to be efficient in the proportion of $\frac{1}{2}$ per cent., but it broke down at $\frac{1}{4}$ per cent. I should, therefore, place the minimum amount which can be safely relied upon to destroy dried

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films of virus and the bacteria of putrefaction (dried upon filtering paper) at 1 per cent.

Sulphurous acid gas.—This agent was tested in various proportions, and was found efficient in the proportion of 1 per cent. for vaccine virus (no experiments made with a smaller amount), and in the proportion of $\frac{1}{2}$ per cent. for bacteria. Like nitrous acid, it broke down at $\frac{1}{4}$ per cent. in experiment No. 40, in which bacteria from putrid urine, dried upon filtering paper, were exposed to its action for six hours.

The conclusion reached is that these three agents, chlorine, nitrous acid (nitrogen dioxide), and sulphurous acid (sulphur dioxide) are reliable disinfectants in the proportion of 1 volume to 100 of air. It is probable that a considerably smaller proportion of the above disinfectants would be efficient in destroying the potency of thin layers of virus in a moist state, or of virus exposed to the action of the disinfectant in an atmosphere saturated with moisture. It was my intention to determine the minimum quantity of each of these agents which could be relied upon to destroy the potency of vaccine virus, both in a dry and in a moist atmosphere, but the difficulty of obtaining unvaccinated persons upon whom to make the trial has prevented me from making further experiments in this direction up to the present time.

Carbolic acid.—The following remarks, quoted from Bulletin No. 47, show the results reached in my experiments with this agent :

The amount of pure acid required to destroy the vitality of bacteria (10 grains, experiment No. 42) is equal to about 17 pounds in a room 12 feet square and 12 feet high (capacity 1,728 cubic feet), and to fulfil the conditions of the experiment in disinfecting on a large scale, it would be necessary to scatter this amount over the floor of a room having these dimensions, and to suspend articles to be disinfected near the floor for at least six hours, care being taken that all apertures were closed so that the fumes of the acid might not escape. Experiment No. 43 shows that four times this amount (68 pounds) of " crude " acid placed upon the floor of a room of the same dimensions would not destroy the vitality of bacteria exposed in the room for six hours. Experiment No. 24 (Bulletin No. 29) shows that an amount of the impure acid equal to 46 fluid ounces volatilized in the same room will not destroy the potency of vaccine virus in a moist state (rubbed up

with glycerine) when the time of exposure is twelve hours. Finally, these experiments show that the popular idea, shared, perhaps, by some physicians, that an odor of carbolic acid in the sick-room, or in a foul privy, is evidence that the place is disinfected, is entirely fallacious, and, in fact, that the use of this agent as a volatile disinfectant is impracticable, because of the expense of the pure acid and the enormous quantity required to produce the desired result.

Recent Experiments with Non-Gaseous Disinfectants.

Having ascertained that I have at hand a ready means of producing a fatal form of septicæmia in the rabbit (see special report to National Board of Health, Bulletin No 44, Vol. II), and that the blood and serum from the sub-cutaneous connective tissue of a rabbit recently dead possesses still greater virulence than the human saliva used in the first instance, the idea occurred to me that this virus could be used to good advantage in further experiments with disinfectants, the test being injection beneath the skin of a healthy rabbit. As the virus so introduced produces death in from twenty-four to forty-eight hours, it is evident that a negative result after treatment with a disinfectant is proof of its power to destroy the virulence of the injected material or, in other words, to disinfect it.

My results have been, in the main, very definite and satisfactory, but my experiments have brought to light certain facts which I did not fully appreciate at the outset, and which to some extent detract from the value of the experiments herein reported.

These facts are :

(a) The action of certain substances may so modify the potency of the virus that the fatal event is postponed from the fifth to ninth day instead of occurring as usual during the first forty-eight hours after injection ; consequently the assumption, upon which I at first acted, that a rabbit which seemed in good health four or five days after an injection, could be placed to the credit of the disinfectant and used for another experiment, cannot be considered a safe one, and it would have been better to allow a longer time to elapse or to have used a fresh rabbit for each experiment. This criticism only applies, however, to a small number of the experiments made, as I have rarely given more than two injections to

the same animal, and in cases where a negative result followed the second as well as the first, the evidence is perfectly definite, the doubt only occurring in those cases in which a fatal result followed a second injection, which might possibly have been due to the previous injection, while credited to the last one made.

The following experiments will serve as examples of this postponement of the fatal event as the result of the action of the disinfectant used :

June 13.—Injected 0.5 c. c. of virus, to which had been added one-tenth of 1 per cent. of iodine (in aqueous solution with potassium iodide).

Result.—Died June 24. *Post-mortem* examination made immediately after death (died in convulsions) showed hemorrhagic extravasations under the skin in vicinity of point of injection, spleen enlarged and dark colored, liver normal, blood from hemorrhagic extravasations under skin and from mesenteric veins (no other examinations made) contains an abundance of micrococci.

Same date (July 13).—Injected 0.5 c. c. of virus containing 10 per cent. of oil of eucalyptus globulus.

Result.—Died June 21 (was killed when evidently on the point of death). Blood drawn into graduate measure coagulates very firmly. Serous discharge from bowels (abundant) contains an abundance of micrococci and other forms of bacteria ; no bacterial organisms found in the blood ; no cellulitis ; liver and spleen normal.

REMARKS IN THIS CASE.—There is no evidence of septicæmia, and it may be that the fatal result was due to the independent action of the oil of eucalyptus, or to some other cause independent of the injection made. Some of the serous discharge (0.25 c. c.) from the bowels of this rabbit was injected into a small rabbit without result. An injection of 0.5 c. c. of blood serum (from graduate measure after retraction of clot) into a small rabbit gave also a negative result.

In the first of these cases the *post-mortem* examination gave evidence of death from septicæmia. In the second the evidence was to the contrary effect ; but it is very evident that either of these rabbits, if made the subject of a second experiment, on the fourth or fifth day after the first injection, although apparently in good health at the time, would have given an uncertain or fallacious result.

The following experiment made at the same time and with the same virus as the preceding is given to show that this virus was reliable :

June 13.—Injected 0.5 c. c. of virus one part and camphor water (aqua camphora of the Pharmacopœia) one part into a small rabbit.

Result. — Death occurred during night of June 15 with the usual symptoms of septicæmia—diffuse cellulitis, enlarged spleen, micrococci in blood, and effused serum in sub-cutaneous connective tissue.

(*b*) Several small rabbits have died without any injection, and from the appearance of the spleen and the presence of the micrococcus in the blood, I have concluded that these were cases of septicæmia, not of traumatic origin, resulting from confinement in cages in which other rabbits, the subjects of my experiments, have died. These septicæmic rabbits have very commonly a serous diarrhœa shortly before death by which their cages and the food remaining in them are soiled, and which contains an abundance of septic micrococci. I have proved experimentally that not only this serous discharge from the bowels but the saliva of an infected animal possesses virulent properties and produces speady death with the usual symptoms. (See special report to National Board of Health, *l. c.*) I suppose, therefore, that these deaths resulted from exposure in infected cages, a supposition which is supported by the observations of Davaine, who affirms that septicæmia may occur among rabbits as an epizootic independently of any wound or contact with other rabbits suffering from septicæmia. (Recherces sur quelques-unes des conditions qui favorisent ou qui empêchent le développement de la septicæmia.   Bull. de l'Acad. de Méd., 2 s., T. VIII, p. 121.)

That these rabbits died from an infectious septicæmia is further proved by the fact that a small quantity of blood from one of them (0.25 c. c.) injected beneath the skin of a large rabbit caused death with the usual symptoms in less than twenty-four hours.

As the companions of these rabbits of the same age (less than two months and weighing about a pound) were subjected to experiment and some died, doubt is thrown upon the result of these experiments and I am obliged to exclude them from my record.

(*c*) The most important source of error, however, and one which must be kept in view in future experiments, is the fact that a pro-

tective influence has been shown to result from the injection of virus, the virulence of which has been modified without being entirely destroyed by the agent used as a disinfectant.

The following experiments will serve as examples of this:

May 24.—Injected into a large rabbit (the subject of a previous experiment, May 13, in which a negative result was noted and in which 0.5 c. c. of virus treated with 1 per cent. of sodium hyposulphite was injected) 1.25 c. c. of virus, not disinfected, from rabbit recently dead.

Result negative.

Same date (May 24).—Injected into large rabbit (subject of previous experiment, May 13, in which 0.15 c. c. of a mixture of virus three parts to alcohol, 95 per cent., one part was injected) 1.25 c. c. of virus not disinfected.

Result.—This animal died June 2, nine days after the injection. *Post-mortem* examination showed the spleen to be small and dark colored; liver contained numerous small abscesses; no diffuse cellulitis; no micrococci in blood. A small quantity of the blood of this animal (0.25 c. c.) was injected into a small white rabbit (weighing about one pound). This animal died June 6. *Post-mortem* examination disclosed limited cellulitis without the presence of micrococci; liver and spleen normal; no micrococci in blood, which contains numerous granular white corpuscles.

REMARKS.—These two animals probably died as the result of the injections made, but they evidently did not die from the malignant infectious septicæmia produced by introduction beneath the skin of an unprotected animal of a small quantity of fluid containing the micrococcus. In the latter case we not only have the marked difference as to date of death, but the characteristic diffuse cellulitis, the greatly enlarged spleen, and the presence of the micrococcus, as distinguishing characteristics. It may be that death in these cases resulted from the poisonous properties of the sepcin, a chemical poison contained in the blood injected, but it is evident that both of the large rabbits previously experimented upon possessed an immunity from the action of the septic micrococcus, or rather that it could not multiply in the bodies of these protected animals, and consequently that death did not result from the infectious form of septicæmia, which has recently been the subject of my studies (*l. c.*). This immunity corresponds with what has been proved to be the case in *charbon*, chicken-cholera,

and pleuro-pneumonia of cattle, in which diseases it has been shown that protective inoculations may be practiced.

In the first case above reported the result was completely negative[1] although the amount of virus injected was considerable (1.25 c. c.), and this virus was proved by comparative experiments to be potent. Other evidence might be adduced in favor of the view that protection results from the effects of inoculations made with virus modified by the action of certain agents; but my object here has simply been to show the importance of considering this possible protective influence of previous injections in making disinfection experiments upon a virus of this character.

My method of collecting virus for disinfection experiments has been to wipe up the bloody serum from the sub-cutaneous connective tissue and from the thoracic and abdominal cavities, after removal of the viscera and puncture of the large veins, with dry cotton, which is then washed out in water. The potency of this diluted virus has been amply proven and, indeed, in every series of experiments made at the same time and with the same material, I have obtained evidence of virulence either from injection of non-disinfected virus as a check experiment, or by the failure of one or more of the substances undergoing trial as disinfectants. Thus in the experiments just reported, the same virus killed a rabbit in less than three days after having been treated with a 4 per cent. solution of magnesia sulphas.

I have not attempted to determine the minimum quantity of virus that would be effectual, but have kept on the safe side by injecting quantities much in excess of the amount required to produce fatal septicæmia. In the experiments of Davaine (*l. c.*), in which the virus in the first instance was obtained from a different source, fatal septicæmia was produced by injections of septicæmic blood in quantities as small as $\frac{1}{500}$ part of a drop.

When we are dealing with a virus of which the virulence depends upon the presence of a living organism capable of self-multiplication in the body of the animal into which it is introduced, it is evident that the question of quantity is quite secondary to

[1] In these experiments no temperature observations have been made, and by a *negative* result failure to kill only is implied. No doubt slight indisposition and a greater or less amount of fever might have been verified in many cases by careful observations, but the object in view rendered such observations unnecessary and want of time rendered them impracticable.

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that of vital activity on the part of the pathogenic organism and vital resistance upon the part of the living tissues of the animal subjected to its action.

It seems probable, in the light of recent experiments, that pathogenic properties in these lowly organisms depend upon rapidity of development and adaptability to conditions such as are found in the interior of the bodies of living animals, and that these qualities may be developed in common and usually harmless bacterial organisms as the result of specially favorable conditions, such as high temperature, abundance of pabulum, &c.

That the virus which has been used in these experiments is capable of producing death in much smaller quantities than those used, is shown by the following experiment:

June 2.—The needle of a hypodermic syringe was dipped into the blood of a septicæmic rabbit just dead, and proved by microscopical examination to contain an abundance of the micrococcus. It was then introduced under the skin of a small rabbit.

Result.—This animal died within 48 hours and presented all the usual appearances of death from septicæmia.

An additional possible source of error will suggest itself as arising from the extreme virulence and the small quantity of material required to produce death. A vey little of this material, not disinfected, adhering to the needle of the hypodermic syringe from one experiment might be the cause of death in a succeeding one and might improperly be ascribed to failure of the disinfectant used in the last experiment. This possibility I have had in view and have carefully guarded against by a thorough disinfecting and cleansing of my syringe after each injection. This has been effected by means of a 10 per cent. solution of carbolic acid or more frequently with diluted sulphuric acid, followed by repeated washings with pure water.

My practice has been to mix the different disinfectants to be used at one time with separate portions of virus, obtained as already described from the cellular tissue and blood-vessels of a rabbit recently dead, in small beakers well cleaned, and to allow a period of twenty minutes to half an hour for the action of the disinfecting agent before making an injection.

Standard solutions of the different substances to be tested were kept in glass-stoppered bottles, and at the outset of my experiments these solutions were made of the strength of 5 per cent.

Solutions of 4 per cent. were afterwards substituted for these because of the greater convenience in reducing the quantity without fractions. Thus one part of virus and one part of a standard 4 per cent. solution gave me the proportion of 2 per cent.; three parts of virus and one of the disinfectant gave the proportion of 1 per cent., &c.

Having fairly stated the possible sources of error in experiments made by this method, I may be permitted to say that I believe my results to be in the main reliable, and that the substances which have best stood the test may be depended upon in practical disinfection in the proportions found to be efficient.

In but a single instance have I had a contradictory result in which the greater quantity failed and the smaller did not. This was in the use of zinc chloride, with which three experiments were made. The rabbit injected with 1 per cent. died, while two others injected with 2.5 per cent. and 0.5 per cent. gave a negative result. To which of the possible causes of error, already pointed out, this contradictory result is due, I am unable to say. The rabbit injected with 1 per cent. may have died from some cause independent of the injection, or from the remote effects of a previous injection, or the rabbit injected with 0.5 per cent. may have been protected by a previous injection. It is evident that in future experiments by this method it will be desirable to use a previously uninjected animal for each experiment.

After this somewhat lengthy preamble, which has seemed necessary, I shall proceed to detail the results of these experiments, placing first those substances which have proved most efficient. For convenience each experiment will be recorded by placing after the name of the substance used the figures representing the proportion in which it was used. Death, or failure to disinfect, is indicated by a full-faced figure representing proportion of disinfectant used. The plain figure indicates a negative result or destruction of virulence by disinfectant (disinfection).

Group 1.

Disinfectants efficient in the proportion of 0.5 per cent. or less.

Iodine (in aqueous solution with potassium iodide), 1.25, 0.5, 0.25, 0.2, **0.1.**[1]

Chromic acid, 1, 0.5, 0.2, 0.1.   (No failure.)

Ferric sulphate, 1.25, 0.5, 0.25, 0.12, **0.12.**[2]

Cupric sulphate, 1, 0.5, 0.25, **0.1.**

Thymol dissolved in alcohol, 1, 0.25, **0.1.**

Caustic soda, 2.5, 1, 0.5, 0.25, **0.2.**

Nitric acid, 1.25, 0.5, 0.25, **0.2.**

Sulphuric acid, 1.25, 0.5, **0.25.**

Ferric sesquichloride, 1, 0.5, **0.25.**

Sodium hyposulphite, 1, 0.5, **0.25.**

Hydrochloric acid, 0.5, **0.25.**

Group 2.

Disinfectants which failed at 0.5 per cent., but proved efficient in proportions below 2 per cent.

Carbolic acid, 2.5, 1.25, **0.5.**

Salicylic acid (as salicylate of soda), 2.5, 1.25, **0.5.**

Zinc chloride, 2.5, **1,** 0.5.[3]

Caustic potash, 2.5, 1, **0.5.**

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[1] In the experiment with 0.1 per cent. the animal did not die until eleven days after the injection; it is, therefore, hardly fair to consider this a failure of the disinfectant, but in the absence of additional experiments I have thought it best to mark this as a failure, and to assume that the limit of safety as to proportion of the disinfectant required has been passed. It was my intention to make a separate series of experiments with potassium iodide for the purpose of ascertaining whether this agent should receive a portion of the credit for the results obtained by the solution used. The scarcity of rabbits has prevented me from making this experiment up to the present time.

[2] Two experiments were made with 0.12 per cent. of ferric sulphate, in one of which the result was negative (disinfection), and in the other the rabbit died (failure to disinfect).

[3] See remarks on page 209 for explanation of this contradictory result.

Iron-alum, 2, 1.

Zinc sulphate, 1.25, **0.5.**

Potassium sulphide (sulphuret), 2, **0.5.**

Tannic acid, 1, **0.5.**

Boracic acid, 2, **1, 1.**

Potassium permanganate, 2, **1, 1.**

Sodium biborate, 2.5, **1.25.**

GROUP 3.

Substances which failed to disinfect in the proportion of 2 per cent.

Potassium nitrate, **4.**

Potassium chlorate, **4.**

Sodium chloride, **2.5.**

Alum, **1.25, 4.**

Lead acetate, **2.**

Magnesia sulphate, **4.**

Glycerine, 25, 12.5, **10.**

Alcohol (95 per cent.), 25, **12.5, 10.**

Camphor water. Equal parts of camphor water and virus were injected with a fatal result.

Pyrogallic acid, **1.**

Oil eucalyptus globulus, **10.** [1]

REMARKS.—It was my intention to make this experimental inquiry as complete as possible before reporting, and to fix definitely the minimum quantity, which may be relied upon to destroy the potency of septic virus (*Micrococcus septicus*), of those substances most commonly used as disinfectants, or prescribed internally, or as lotions, with a view to their antiseptic action; also to determine the time during which the septic virus will retain its

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[1] The rabbit injected with one part of oil eucalyptus to nine of virus did not die until eight days after the injection, and the *post-mortem* examination showed that it did not die of septicæmia. This cannot, therefore, be fairly considered a failure to disinfect, and further experiments will be required to determine the value of this agent, which is especially interesting just now from the fact that Lister is using it in his antiseptic dressings to wounds.

potency in a dry state; the effect of gaseous and volatile disin-
fectants upon the dried virus, both in a dry and moist atmos-
phere; the comparative value of various proprietary disinfectants
now in the market; the thermal death-point of *Micrococcus septi-
cus*, &c.

It will be seen that I have fallen far short of the accomplish-
ment of this purpose, but I have thought it best to report what
has already been accomplished, as practical sanitarians may obtain
some hints of value from the experiments recorded, and it is very
uncertain when I will be able to resume my experiments, which I
have been obliged to discontinue on account of the pressure of
other duties and the difficulty of obtaining rabbits for experi-
mental purposes.

OBSERVATIONS ON THE DIRECT INFLUENCE OF VARIATIONS OF ARTERIAL PRESSURE UPON THE RATE OF BEAT OF THE MAMMALIAN HEART. By H. NEWELL MARTIN, M. A., M. D., D. Sc. With Plate XV.

THE earliest observations on this subject, so far as I know, were made by Marey (Recherches sur le pouls au moyen d'un nouvel appareil enrégistreur. *Mémoires de la Société de Biologie*, 1859); but as the extrinsic cardiac nerves were not divided in his experiments, and a rise of blood pressure is now known to stimulate the medullary cardio-inhibitory and accelerator nerve centres, the results obtained by him give really no information as to the direct influence of increased aortic tension upon the rate of the heart's beat. Since then others have experimented, previously dividing the extrinsic cardiac nerves, Ludwig and Thiry in 1864 (*Sitzb. d. Akad. d. Wissensch. zu Wien*) leading the way, but the general result is that the matter has been left in a highly unsatisfactory state. Some find that variations of arterial pressure have no effect on a heart whose venous connections with other parts of the body have been severed; others that arterial pressure and pulse rate rise and fall together; others that the pulse quickens when arterial tension is lowered and *vice versa*. Finally, Tschirjew (*Arch. f. Anat. u. Physiologie, Jahrgang* 1877, p. 116), the latest writer on the subject, finds all of the above effects in different cases: as the result of an extensive series of experiments he comes to the conclusion that after section of all the extrinsic heart nerve paths, "any considerable and rapid elevation of blood pressure may directly stimulate either the inhibitory apparatus in the heart, or its motor ganglia, and the pulse rate accordingly be increased or diminished, or in more rare cases remain unaltered." Such contradictory results obtained by a number of competent workers lead naturally to the suspicion that some error is involved in the methods of experiment employed; the nature of this error is not, I think, far to seek. The methods used to vary arterial pressure have been such as cause variations also in several other conditions which

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either are known to influence the heart, or may possibly do so; nevertheless all these secondary actions have been unheeded: their relative prominence in any given experiment has not been noted, and any change in the pulse rate has been ascribed solely to the changed arterial pressure. Under such circumstances it need cause no surprise that very inconsistent results should be obtained.

The higher aortic pressure is, the more force must be expended by the left ventricle in forcing open the semilunar valves; that is to say, the higher will be intraventricular systolic pressure. It is this influence only of increased aortic pressure which should be meant when its direct action upon the cardiac rhythm is spoken of; and to get pure results all other consequences of increased arterial tension which may influence the heart's rate of beat must be eliminated. This, however, has not been the case in any series of experiments with which I am acquainted.

Arterial pressure has commonly been increased by clamping the descending aortic, either in the thorax or abdomen. When this is done, however, we alter several other things in addition to arterial pressure—

(1.) The amount of blood returned to the right auricle in a given time is almost certainly altered, and therefore the rate of filling of the heart during diastole.

(2.) The pressure under which venous blood enters the right auricle is probably changed, and therefore intracardiac pressure at the end of the diastole.

(3.) The temperature of the blood returned to the heart by the systemic veins and, as a consequence, of the heart itself, is altered. The blood returned to the right auricle by the inferior cava is known to be warmer than that returned by the superior cava, which has not flowed through the hot abdominal organs. When the aorta is clamped the heart gets only the cooler superior cava blood, as the capillary tracts tributary to the inferior cava are no longer supplied with blood.

(4.) It is known that very slight chemical changes in the blood profoundly influence the heart's beat. To quote no other instance, Gaule has shown that the heart of a frog previously kept in the cold and exhibiting deficient functional power, may be restored to full vigor by circulating through it the extract of the heart of a frog kept previously at a higher temperature. Blood in its flow through the abdominal organs experiences important chemical

changes entirely differing from any undergone in other regions of the body. If, therefore, we circulate blood through head, neck and fore limbs only, and return it again and again to the heart without exposing it to the action of kidneys, spleen and liver, we very soon have a liquid to deal with which is essentially different from that which flowed through the heart before the aorta was ligated.

Of course when the arterial pressure is lowered by opening the previously clamped aorta all of the above possible disturbing actions occur in the opposite direction.

Another method which has been employed to raise arterial pressure is to inject blood from another animal into the carotid of the animal experimented upon. This also involves several possible sources of error. (1) Venous inflow during cardiac diastole is almost certainly changed. (2) Venous pressure and, therefore, intracardiac diastolic pressure are probably altered. (3) The injected blood may differ chemically from that already in the vessels, and directly act upon the heart. (4) Unless extreme care be taken the temperature of the injected blood will be less or greater than that of the already circulating blood, and will alter the temperature and, therefore, the rhythm of the heart. To the above objections it may be added that only slight increase of arterial pressure can be brought about in this way ; as is proved by Worm Müller's experiments. (*Arbeiten aus d. physiol. Anstalt zu Leipzig*, 1873).

When blood pressure is lowered by bleeding, diastolic inflow and pressure are altered, as well as arterial pressure; and also probably the chemical metabolisms experienced by the blood in its flow through different organs.

As some one, at least, of the above secondary influences has been present in all previous experiments as to the influence of variations of arterial pressure upon the pulse rate, it is clear that none of these experiments, interesting and important as their results are in many cases, are really capable of affording an answer to the question in hand, viz: what is the influence, if any, pure and simple, of increased aortic pressure (*i. e.* of increased systolic pressure within the left ventricle) on the pulse rate. It is, therefore, not necessary to consider in detail the experiments of previous writers. All are vitiated more or less by secondary changes

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which have occurred along with the variations of arterial pressure; and the number of these possible complications, and their varying degree in different experiments, affords a sufficient explanation of the contradictory results obtained.

As regards the frog's heart, there is more agreement between observers, and the experimental conditions have usually been more satisfactory. Usually the auricle is supplied steadily with liquid of constant composition and at constant pressure from a Marriott's flask; but even here, so far as I know, the arterial cannula has always been inserted into the ventricle and, therefore, beyond the semilunar valves. As a necessary consequence of this, not only systolic ventricular pressure (which normally is the thing changed by varied arterial pressure), but also diastolic intraventricular pressure has been varied. I accordingly suggested to two of my pupils that they should undertake a fresh examination of this question by better methods, on the hearts of frogs and chelonia. Some results of their work will be found on subsequent pages of the present number of this Journal.

The question involved is clearly one of great importance. In almost every experiment relating to cardiac physiology arterial pressure is altered: and it is essential to know exactly the direct influence of this factor on the heart, before further conclusions can be legitimately arrived at. I have, therefore, lately carried out a large number of experiments as to the direct influence of variations of arterial pressure upon the pulse, making use of the dog's heart completely isolated physiologically from every other organ, but the lungs: the method of isolation, which essentially consists in closing the whole systemic circulation except that through the coronary vessels of the heart itself, was described by me in the last number of this Journal (Vol. II, No. 1, p. 119); as the apparatus has since been modified only in some points of detail, I here reproduce, as Plate XV, the figure used in illustrating the previous paper, in order to assist in the description of my more recent experiments.

The right and left carotid arteries, o and r, have cannulas placed in them, the right subclavian, w, is ligatured, and a cannula is put in the left subclavian, m. Then the aorta is ligated immediately beyond the origin of the left subclavian: the vena cava inferior and the azygos vein are tied, and a cannula put in the superior cava. Fresh defibrinated strained and warmed blood is

now run in by the superior cava; at the same time the cannula on
the right carotid is opened, and blood drawn from it until there is
reason to believe that all the blood originally in the heart and
lungs of the animal has been washed out; the carotid is then
again clamped, and the superior cava a few seconds later, when
the heart and lungs have been tolerably well filled with blood.
The animal is then transferred to the warm moist chamber, K,
the cannula of the superior cava is connected with one of the Mar-
riott's flasks, 27 or 28, from which a nutrient liquid is sent into
the heart under a uniform pressure, which in the experiments
described below was that exerted by a column of blood 10 centi-
metres in height.   The left carotid, o, is connected with the out-
flow tube, 21, and the cannula in the subclavian with a mercurial
manometer, 26, the pen of which writes on the paper of a kymo-
graphion in the usual manner.   As soon as one Marriott's flask is
empty its connection with the heart is shut off, and that of the
other (which has been meanwhile closed) is freed by opening the
proper one of the clamps, 1 or 2, and closing the other.   The
nutrient liquids employed in the experiments below described were
(1) fresh defibrinated strained dog's blood ; (2) the same diluted
with an equal bulk of 0.5 per cent. solution of sodium chloride
in distilled water.   I may here state that in other cases I have
used with success (3) defibrinated dog's blood with one-third its
bulk of 0.7 per cent. sodium chloride solution; and (4) defibri-
nated calf's blood.

Under these conditions almost all of the ordinary collateral
results of increased or lowered arterial pressure can be elimi-
nated.   By closing more or less completely the stop-cock, 22,
arterial pressure can be raised ; by opening the stop-cock wider
it can be diminished.   Meanwhile rate of supply to the right
auricle, the temperature of the liquid sent into it, and the compo-
sition of this liquid are unvaried ; all these disturbing elements
are thus got rid of.   I have said above that "almost" all secon-
dary effects can be eliminated ; the *almost* is due to the varied
coronary circulation ; when aortic pressure is high this must be
greater than when that pressure is low ; so far I see no method of
eliminating this possible source of error ; but in recent years much
evidence has been accumulated to shew that if the flow of blood
through an organ is sufficient to nourish it (*i. e.*, does not fall
below the starvation limit), and is under a lower pressure than
such as ruptures the vessels or otherwise mechanically impedes

the action of the organ, there is much reason to believe that varia-
tions in blood supply have no immediate influence on its functional
activity. The experiments detailed below give further support to
this view: as will be seen, variations of arterial pressure ranging
between 25 and 150 mm. of mercury have no influence whatever
upon the heart's rhythm, although considerably more blood must
flow through the coronary system under the higher than under the
lower pressure.

In the experiments described below the heart was always left in
the warm chamber at least half an hour before observations were
made, and longer if the thermometer did not shew that the tem-
perature was then uniform and had been for some five or ten
minutes. The animals during the isolation of the heart were
sometimes placed under the influence of morphia, sometimes of
curari, and sometimes of chloroform; these various agents were
used to eliminate chances of error due the possible toxic action
of any one of them on a regulatory mechanism in the heart,
though when fresh unpoisoned defibrinated blood is run for hours
through the heart after its isolation, there can be little doubt
that any poison absorbed by the organ during the preliminary ob-
servation is thoroughly washed out. The animals used were small
dogs, weighing from 6 to 7.5 kilos. Uniform artificial respiration
was kept up by means of a small water engine.

When temperature had become constant, the connection between
a full Marriott's flask (containing about 700 c. c. of liquid) and
the heart was opened. A minute or two was allowed to elapse, to
get a steady inflow current; then arterial pressure was raised by
partially closing the stop-cock, 22, or lowered by opening it wider.
Tracings were taken for from two to six minutes with arterial pres-
sures varied in this way; then the observation ceased. Mean-
while the other Marriott's flask was filled; and after some minutes
another observation was made while it was connected with the
heart; and so on, so often as seemed desirable. In all cases the
experiment came to an end long before the heart shewed signs of
abnormal or irregular action; indeed in most instances it was sub-
sequently used for preliminary observations on the influence of
other conditions, as varied venous pressure or varied temperature
on the pulse rate.

The results arrived at may be summed up as follows:

1. *When the pressure under which blood of uniform temperature
and composition is steadily supplied to the right auricle does not*

exceed that due to a column of blood ten centimetres in height, no variation of arterial pressure which can be brought about by opening or closing more or less completely the outflow stop-cock, has any influence whatever on the rhythm of a heart isolated from all other organs of the body except the lungs, provided arterial pressure be not kept at a very low level for a considerable time. In other words, within very wide limits, changes in arterial pressure have no influence whatever upon the pulse rate.

2. *If the outflow stop-cock be widely opened and arterial pressure lowered to less than twenty millimetres of mercury, this has no direct influence on the pulse rate; but it has probably an indirect influence. For a minute or more the heart beats recur at the same intervals, but after that time, if the low pressure be still maintained, the pulse sometimes becomes slower, probably from deficient nutrition of the heart dependent on insufficient flow through the coronary vessels.*

3. *If the pressure at which venous blood enters the right auricle be considerable (due to a column of blood forty centimetres in height), and if simultaneously the arterial exit be greatly narrowed by closing the outflow stop-cock, then arterial pressure at first rises greatly without any alteration in the pulse rate; but ultimately attains a very high level at which the cardiac rhythm becomes extremely irregular. Beats occur which somewhat resemble those produced by feeble pneumogastric stimulation. If the arterial resistance be now diminished, markedly dicrotic beats occur for some twenty or thirty seconds, until arterial pressure again falls to a normal level, when the original pulse rate is resumed. The conditions when the irregular beats are observed are clearly pathological: a filling of the heart under a pressure in the venæ cavæ equal to forty centimetres of blood (twenty-nine millimetres of mercury) probably never occurs normally combined with great arterial resistance.*

In the present article I shall confine myself to what may be called normal variations of arterial pressure, that is to say, for small dogs, variations between 25 and 160 millimetres of mercury. The result under the above heading 2 is undoubtedly abnormal, and due to commencing death of the heart; and the results indicated under number 3 are probably due either to the reception by the left ventricle in each diastole of more blood than, under the resistance opposed to it, it can pump out in one systole, or to a direct stimulation of inhibitory mechanisms in the heart by the pathological pressure within the ventricle. This irregular beat

with very great arterial resistance has been noted by Haidenhain, and I may here state that Knoll's opinion that it really means not a slowed heart beat, but a quick irregular beat which the manometer does not properly record, is incorrect; direct observation of the exposed heart is conclusive as to the fact that the beats are not quick and irregular, but really slow, and frequently dicrotic.

On the results numbered 2 and 3 above I desire to make further observations before publishing detailed conclusions. Hitherto so soon as I have observed indications of them I have at once raised or lowered arterial pressure so as to prevent death or injury to the heart. As regards point 1, the three tables below speak for themselves. They are selected from a dozen experiments which are perfectly concordant, and they have been so selected that a different drug was given to the dog during the preliminary operation of isolating the heart in each case. The venous inflow was always so proportioned to the resistance to arterial outflow that pressure in the subclavian during the intervals between any two observations was kept at a point from which arterial pressure could be considerably raised without the variation passing beyond a physiological limit; but at the same time, a pressure sufficient to keep the heart in a functional condition for a long time.

Venous pressure in all the experiments recorded below was that due to a column of nutrient liquid (defibrinated dog's blood, or the same diluted with an equal volume of sodium chloride solution) ten centimetres in height, or very near that; it is not well practicable to measure exactly in every experiment the difference in level between the cannula in the superior cava and the lower end of the tube for the entry of air into the Marriott's flask; but errors of a few millimetres in this regard are of no importance: so long as the pressure is constant during an observation a knowledge of its absolute amount within 5 or 6 millimetres of blood is of no consequence.

The tables are constructed as follows: Temperature in the moist warm chamber having become constant, the kymographion was started and tracings taken for from two to seven minutes. During this time the stop-cock, 22, was opened wider, or more closed, or opened and then closed, or *vice versa*, and consequently arterial pressure was altered. A number of such observations having been made the tables were constructed from the tracings obtained: suppose the time to be 2 h., 20′, 10″, then arterial pressure is

measured at that time and at 2 h., 20', 20''. Half the sum of these
is taken as the mean pressure during the intervening ten seconds.
The pulse rate is counted for this ten seconds, multiplied by 6, and
the product given as the rate of heart beat per minute, with the
mean arterial pressure obtained as above. So far as absolute results
are concerned, it is seen that the mean arterial pressure arrived
at in this way is open to some error, and had changes in it been
accompanied by changes in the pulse rate, more accurate methods
of arriving at the true mean arterial pressure during each ten
seconds would have to be employed. But as very great variations
of mean arterial pressure were used and as the experiments shew
that none of them, within the limits described above as physio-
logical, cause any change in the rate of the heart's beat, it is clearly
unnecessary to resort to planimetry or other troublesome methods
so as to avoid possible errors of a few millimetres in the measure-
ments. When gross variations of arterial pressure from 30 to 150
mm. of mercury cause no change, it is not worth while to spend
time in endeavoring to exclude possible errors of ten or even
fifteen millimetres of mercury pressure; and the possible limits
of error in my measurements never reached the less of those
quantities. When the lungs are kept well extended and the arti-
ficial respiration apparatus works with tolerably slow powerful
blasts, marked respiratory waves are seen on the tracings of arte-
rial pressure, unless this fall to 50 millimetres of mercury or
thereabouts, when they disappear. As these rhythmic rises and
falls of arterial pressure render it more difficult to correctly arrive
at the mean pressure, I have usually eliminated them by arranging
my water engine so as to work with rapid short strokes; then res-
piratory variations of arterial pressure entirely disappear from the
manometer tracings.

In the experiments recorded below the heart had been physio-
logically isolated from all other organs but the lungs for some
considerable time before the recorded observations were made;
the muscles of the body in general were often already in marked
rigor before the first observation was made and always long before
the last. When the words "no record" appear in the details of
an observation, some one or more of the pens was not writing, so
that either time, pressure, or pulse rate, could not be determined.
The temperature given is that of the warm chest in which the
animal lay.

Experiment A.

October 13, 1881. Small dog, narcotised with morphia during the operation of isolating the heart. Nutrient liquid 1,400 cub. cent. of defibrinated dog's blood drawn from two other animals. Arterial pressure measured in left subclavian. Heart isolated and animal put in warm chamber at 4 h. 10′, P. M.

Observation.	Time.	Temperature in degrees C.	Arterial Pressure in mm. of mercury.	Pulse Rate per minute.
I.	4 h. 44′ 00″	37°	137	147
	" 10		134	147
	" 20		131	146
	" 30		132	147
	" 40		116	147
	" 50		89	147
	4 h. 45′ 00″		74	147
	" 10		83	150
	" 20		109	147
	" 30		124	147
	" 40		134	150
	4 h. 46′ 00″		149	150
	" 10		142	149
	" 20		120	147
	" 30		98	147
	" 40		83	150
	" 50		99	147
II.	4 h. 58′ 50″	37°	133	147
	4 h. 59′ 00″		134	149
	" 10		139	147
	" 20		143	150
	" 30		144	150
	" 40		142	149
	" 50		138	149
	5 h. 00′ 00″		136	148
	" 10		129	150
	" 20		104	150
	" 30		82	150
	" 40		87	150
	" 50		117	151
	5 h. 01′ 00″		123	148
	" 10		129	151
	" 20		133	150
	" 30		130	150
	" 40		110	150
	" 50		90	151

EXPERIMENT A.—*Continued.*

Observation.	Time.	Temperature C.	Arterial Pressure.	Pulse Rate.
III.	5 h. 17′ 00″	37°	112	150
	" 10		119	150
	" 20		102	150
	" 30		80	150
	" 40		87	No record.
	" 50		100	150 (?)
	5 h. 18′ 00″		108	No record.
	" 10		114	150
	" 20		119	150
	" 30		125	No record.
	" 40		126	151
	" 50		112	150
	5 h. 19′ 00″		89	150
IV.	5 h. 29′ 40″	37°	80	150
	" 50		81	153
	5 h. 30′ 00″		80	156
	" 10		82	150
	" 20		93	156
	" 30		104	153
	" 40		110	153
	" 50		112	156
	5 h. 31′ 00″		111	153
	" 10		112	150
	" 20		99	150
	" 30		80	156
	" 40		82	156
	" 50		93	153
	5 h. 32′ 00″		102	156
	" 10		100	156
	" 20		86	156
	" 30		85	150
	" 40		97	156
	" 50		102	152

In observation I, arterial pressure varied between 74 and 149 millimetres of mercury (101 per cent.) and the pulse rate between 147 and 150 per minute (2 per cent.). In observation II, arterial pressure varied between 82 and 144 millimetres of mercury (75.6 per cent.) and the pulse rate between 147 and 151 per minute (2 per cent.). In observation III, arterial pressure varied between 80 and 126 millimetres of mercury (57.5 per cent.) and

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the pulse rate between 150 and 151 per minute (0.66 per cent.).
In observation IV, arterial pressure varied between 80 and 112
millimetres of mercury (40 per cent.) and the pulse rate between
150 and 156 per minute (4 per cent.).

EXPERIMENT B.

October 15, 1881.   Small dog, curarised during the preliminary
operation.   Nutrient liquid 1,350 cub. cent. of defibrinated dog's
blood taken from two other animals.   Arterial pressure measured in
left subclavian.   Operation completed and animal placed in warm
chest at 1 h. 50', P. M.

Observation.	Time.	Temperature in degrees C.	Arterial Pressure in mm. of mercury.	Pulse Rate per minute.
I.	2 h. 17′ 50″	34.5°	53.5	120
	2 h. 18′ 00″		78.5	120
	" 10		116.5	120
	" 20		No record.	No record.
	" 30		No record.	No record.
	" 40		86	120
	" 50		75	120
	2 h. 19′ 00″		69	120
	" 10		66	120
	" 20		80.5	122
	" 30		102.5	122
	" 40		114	121
	" 50		121	120
II.	2 h. 44′ 00″	35°	53	117
	" 10		57.5	117
	" 20		84	117
	" 30		117	123
	" 40		136	114
	" 50		145	118.5
	2 h. 45′ 00″		104	114
	" 10		67	118.5
	" 20		51	114
	" 30		49	117
	" 40		49	117
	" 50		35	117
	2 h. 46′ 00″		27	114
	" 10		25	117
	" 20		23	117
	" 30		22	114

EXPERIMENT B. OBSERVATION II.—*Continued.*

Observation	Time.	Temperature C.	Arterial Pressure.	Pulse Rate.
II.	2 h. 46′ 40″	35°	22.5	114
	" 50		22.5	113
	2 h. 47′ 00″		21	111
	" 10		20	111
	" 20		25	114.5
	" 30		45	110
III.	2 h. 54′ 50″	35°	148	108
	2 h. 55′ 00″		116	108
	" 10		78	112
	" 20		56	108
	" 30		43	109.5
	" 40		38	108
	" 50		41	108
	2 h. 56′ 00″		51	108
	" 10		57	108
	" 20		89	112
	" 30		131	111
	" 40		143	110
IV.	3 h. 27′ 40″	35°	72.5	99
	" 50		87.5	102
	3 h. 28′ 00″		99.5	100
	" 10		117.5	99
	" 20		128	102
	" 30		140	103
	" 40		No record.	No record.
	" 50		No record.	No record.
	3 h. 29′ 00″		No record.	No record.
	" 10		91	102
	" 20		73	102
	" 30		59	102
	" 40		43	102
	" 50		44	102
V.	3 h. 31′ 20″	35°	63	98
	" 30		81	102
	" 40		98	98
	" 50		110	99
	3 h 32′ 00″		119	100
	" 10		No record.	No record.
	" 20		No record.	No record.
	" 30		No record.	No record.
	" 40		No record.	No record.

Observation	Time.	Temperature C.	Arterial Pressure.	Pulse Rate.
V.	3 h. 32′ 50″	35°	127	101
	3 h. 33′ 00″		106	102
	" 10		70	102
	" 20		54	101
	" 30		47	99
	" 40		55	100.5
	" 50		72	103
	3 h. 34′ 00″		89	102
	" 10		104.5	102
	" 20		112.5	101.5
	" 30		122	103
	" 40		130	102
	" 50		131	103
	3 h. 35′ 00″		111	104
	" 10		80	102
	" 20		65	100
	" 30		40	102
	" 40		51	102
	" 50		56	102
VI.	3 h. 40′ 55″	35°	50.5	102
	3 h. 41′ 05″		58.5	101
	" 15		64	102
	" 25		64	102
	" 35		66	102
	" 45		80	102
	" 55		100	102
	3 h. 42′ 05″		114	102
	" 15		101	102
	" 25		71	102
	" 35		61	102
	" 45		75.5	103
	" 55		98.5	102
	3 h. 43′ 05″		112	104
	" 15		102	103
	" 25		74	102
	" 35		56	101
	" 45		45	100
	" 55		33	102
	3 h. 44′ 05″		25	102
	" 15		23	102
	" 25		22	104
	" 35		18.5	102
	" 45		17.5	103

In observation I of the above experiment arterial pressure varied between 53.5 and 116.5 millimetres of mercury (117 per cent.) and the pulse between 120 and 122 per minute (1.6 per cent.). In observation II, arterial pressure varies between 20 and 145 millimetres of mercury (625 per cent.) and the pulse rate between 110 and 118.5 per minute (nearly 8 per cent.); this it will be seen on closer examination is one of the cases above referred to, which lead to the suspicion that a continued arterial pressure (as measured in the subclavian) of less than 30 millimetres of mercury is insufficient to nourish the heart and leads to a slowing of its beat. Arterial pressure was kept below this limit for nearly one and a half minutes, and the pulse rate fell from 117 to 110. In observation III, arterial pressure varies between 38 and 148 millimetres of mercury (290 per cent.) and the pulse rate between 108 and 112 per minute (3.6 per cent.). In observation IV, arterial pressure varies between 43 and 140 millimetres of mercury (225.5 per cent.) and the pulse rate between 99 and 103 per minute (4 per cent.). In observation V, arterial pressure varies between 40 and 111 millimetres of mercury (177.5 per cent.) and the pulse rate between 100 and 104 per minute (4 per cent.). In observation VI, arterial pressure varies between 17.5 and 114 millimetres of mercury (551.5 per cent.) and the pulse rate per minute between 100 and 104 (4 per cent.).

EXPERIMENT C.

October 26, 1881. Small dog, anæsthetised by chloroform during the operation of isolating the heart. Nutrient liquid 800 c. c. of defibrinated dog's blood mixed with 800 c. c. of 0.5 per cent. solution of pure sodium chloride in distilled water. Heart isolated and animal placed in warm chest at 12 h. 50′, P. M. When the series of observations detailed below was concluded the heart was still in good condition and was used for two hours for other experiments.

Observation.	Time.	Temperature in degrees C.	Arterial Pressure in mm. of mercury.	Pulse Rate per minute.
I.	1 h. 23′ 10″	37°	29	102
	" 20		30	103
	" 30		30	102
	" 40		30	102

Observation.	Time.	Temperature C.	Arterial Pressure.	Pulse Rate.
I.	1 h. 23′ 50″	37°	33	103
	1 h. 24′ 00″		40	102
	" 10		46	103
	" 20		51	102
	" 30		59	102
	" 40		63	103
	" 50		56	101
	1 h. 25′ 00″		46	102
	" 10		40	102
	" 20		35	103.5
	" 30		42	102
	" 40		58	103
	" 50		70	102
	1 h. 26′ 00″		79	105
	" 10		80	104.5
	" 20		No record.	No record.
	" 30		40	105
	" 40		36	105
	" 50		26	105
II.	1 h. 33′ 20″	37°	40	100
	" 30		42	101
	" 40		43	102
	" 50		44	102
	1 h. 34′ 00″		37	102
	" 10		30	102
	" 20		25	102
	" 30		25	101
	" 40		28	101
	" 50		29	101
	1 h. 35′ 00″		28	102
	" 10		27	102
	" 20		29	101
	" 30		39	100.5
	" 40		52	102
	" 50		63	102
	1 h. 36′ 00″		72	102
	" 10		56	102
	" 20		32	102
	" 30		29	101.75
	" 40		41	101
	" 50		58	102
	1 h. 37′ 00″		68	102
	" 10		78	103
	" 20		87	103

EXPERIMENT C. OBSERVATION II.—*Continued.*

Observation.	Time.	Temperature C.	Arterial Pressure.	Pulse Rate.
II.	1 h. 37' 30''	37°	93	105
	" 40		98	102
	" 50		101	102
	1 h. 38' 00''		103	102
	" 10		88	102
	" 20		53	102
	" 30		29	102
	" 40		25	101
	" 50		25	100.5
	1 h. 39' 00''		24	100.5
	" 10		24	102
	" 20		26	102
	" 30		27	102
	" 40		26	100.5
	" 50		28	100.5
III.	1 h. 57' 30''	37°	28	96
	" 40		38	94
	" 50		24.5	97
	1 h. 58' 00''		29.5	95
	" 10		33	96
	" 20		25	96
	" 30		14.5	99
	" 40		12	95
	" 50		14.5	96
	1 h. 59' 00''		20	96
	" 10		24.5	96
	" 20		29	96
	" 30		34	98
	" 40		37.5	99
	" 50		30	96
IV.	2 h. 02' 10''	37°	51	100
	" 20		54	100.5
	" 30		64	100.5
	" 40		76	102
	" 50		87	102
	2 h. 03' 00''		94	102
	" 10		89	103
	" 20		56	105
	" 30		30	102
	" 40		37	102
	" 50		54	105
	2 h. 04' 00''		70	108
	" 10		81	104

EXPERIMENT C.　OBSERVATION IV.—*Continued.*

Observation.	Time.	Temperature C.	Arterial Pressure.	Pulse Rate.
IV.	2 h. 04′ 20″	37°	89	104
	"　30		95	106
	"　40		99	105
	"　50		106	105
	2 h. 05′ 00″		81	105
	"　10		39	105
	"　20		21	105
	"　30		24	104
	"　40		35	105
	"　50		50	108
	2 h. 06′ 00″		64	105
	"　10		77	108
	"　20		88	109
	"　30		81	110
	"　40		48	108
	"　50		23	108
	2 h. 07′ 00″		27	108
	"　10		42	107
	"　20		59	109
	"　30		73	110
	"　40		83	111
	"　50		77	109
	2 h. 08′ 00″		No record.	No record.
	"　10		19	109
	"　20		18	109
	"　30		19	109
V.	2 h. 17′ 20″	37°	25	105
	"　30		26	108
	"　40		29	105
	"　50		33	105
	2 h. 18′ 00″		40	106
	"　10		49	106
	"　20		53	106
	"　30		57	106.5
	"　40		63	106.5
	"　50		68	106.5
	2 h. 19′ 00″		71	106.5
	"　10		72	106
	"　20		73	106.5
	"　30		76	108
	"　40		77	106
	"　50		78	107
	2 h. 20′ 00″		77	105
	"　10		53	105

EXPERIMENT C. OBSERVATION V.—*Continued.*

Observation.	Time.	Temperature C.	Arterial Pressure.	Pulse Rate.
V.	2 h. 20′ 20″	37°	29	105
	" 30		23	105
	" 40		22	105
	" 50		24	105
	2 h. 21′ 00″		30	105
	" 10		39	105
	" 20		45	106.5
	" 30		53	105
	" 40		· 61	106.5
	" 50		66	106.5
	2 h. 22′ 00″		71	106.5
	" 10		No record.	No record.
	" 20		No record.	No record.
	" 30		76	106.5
	" 40		69	106.5
	" 50		46	106.5
	2 h. 23′ 00″		26	106.5
	" 10		22	106.5
	" 20		21	106.5
	" 30		20	106.5

In observation I of the above experiment, arterial pressure varied between 26 and 80 millimetres of mercury (207 per cent.) and the pulse between 101 and 105 per minute (4 per cent.). In observation II arterial pressure varied between 24 and 103 millimetres of mercury (329 per cent.) and the pulse rate between 100 and 105 per minute (5 per cent.). In observation III, arterial pressure varied from 12 to 38 millimetres of mercury (216.5 per cent.) and the pulse rate from 94 to 99 per minute (5 per cent.). In observation IV, arterial pressure varied between 18 and 106 millimetres of mercury (863 per cent.) and the pulse rate between 100 and 111 per minute (11 per cent.). In observation V, arterial pressure varied between 20 and 78 millimetres of mercury (290 per cent.) and the pulse rate between 105 and 108 per minute (less than 3 per cent.).

A critical examination of the preceding tables will, I think, shew conclusively that variations in arterial pressure within the limits indicated in them have no influence on the pulse rate of the

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isolated dog's heart. In the great majority of cases the variations
in the pulse rate fall clearly within the limits of error of the
experiment (2-3 per cent.), while arterial pressure is greatly varied.
Eliminating the obviously exceptional observations II, Expt. B,
and IV, Expt. C, the average variation of arterial pressure in an
observation was 204 per cent., and the average variation in the
pulse rate 3.3 per cent.

That the possible sources of error will readily account for the
pulse changes in most cases is clear—when it is remembered
(1) that a mistake of one-sixth of a beat in counting out the pulse
in any period of ten seconds appears in the tables as an error of
one beat per minute; (2) that the temperature of the air pumped
through the lungs and influencing the temperature of the blood
was often unavoidably altered during the course of an observation
as the doors of my present experiment room, which unfortunately
is somewhat of a thoroughfare, were opened by passers-by from
time to time. The latter influence is of great importance, as
experiments which I hope shortly to publish, have proved that
the dog's heart is, so far as its rhythm is concerned, extremely
sensitive to slight variations in temperature.

Whatever the cause of the slight pulse-rate changes observed
may be, it is at least clear that they are not dependent on varied
aortic pressure, for there is no possible relationship, direct or
inverse, to be detected between the two, when the whole series of
observations is examined. In most cases great variations of arte-
rial pressure are seen to occur without any change in the pulse
rate, and then, a little later in the same observation perhaps, the
pulse alters two or three beats a minute without any considerable
simultaneous change in arterial pressure.

If the relationship between pulse rate and arterial pressure were
invariable, even 3.3 per cent. of variation in the pulse per minute
might clearly be significant: but as there is no such constant rela-
tionship, and the known sources of error fully account for such
pulse-rate variations as were observed, they obviously mean nothing
in this connection: and we may safely conclude that *within the
limits of aortic pressure indicated by pressures varying between 25
and 140 millimetres of mercury in the subclavian, no change of
pressure has any direct action upon the rate of beat of the isolated
heart of the dog.*

Before concluding it is my duty and pleasure to acknowledge the willing and skilful assistance in the execution of my experiments rendered to me by Mr. H. H. Donaldson and Mr. Mactier Warfield, who not only undertook the tedious task of getting ready the apparatus for each experiment, but gave me most important help in carrying it through.

THE INFLUENCE OF CHANGES OF ARTERIAL PRESSURE UPON THE PULSE RATE, IN THE FROG AND THE TERRAPIN.

By WM. H. HOWELL, A. B., and MACTIER WARFIELD, A. B. With Plate XVI.

At the request of Professor Martin we undertook some experiments upon this subject, to see if the same results would be obtained from these animals, as were obtained with the isolated mammalian heart.

We used substantially the same method as that employed by Professor Martin in his experiments, described in the preceding paper, keeping the venous pressure constant and varying only the pressure in the outflow tube connected with the aorta, in a way to be described presently.

As far as we have seen, no one has hitherto, in experiments on these animals with regard to the effects of changes of blood pressure, varied the arterial pressure alone.

Most of the work on the subject has been done with variations of diastolic pressure. Luciani[1] tried also the effects of variation of systolic pressure. His method, however, did not furnish the conditions which prevail in normal variations of arterial pressure. He states that his apparatus was not suitable for studying the effects of such changes, and does not give his results. With regard to diastolic pressure, he says "that neither the frequency nor the absolute height of the pulse was actually changed, when the pressure (in the frog) was raised from 4 mm. to 13 mm. of mercury."

Tschiriew[2] studied the effects of variations of both systolic and diastolic pressure in the heart of the frog. He gets the same result in both cases, viz: a quickening of the pulse rate with increased pressure.

He does not describe his method of varying systolic pressure, but it is evident that it was not the effects of varied arterial pres-

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[1] Luciani. Eine periodische Function des isolirten Froschherzens. Ludwig's Arbeiten, 1872.

[2] Tschiriew. Arch. f. (Anat. u.) Physiol., 1877.

sure alone that he got, since his arterial cannula was thrust beyond the semilunar valves into the ventricular cavity and hence the increased aortic pressure must have acted upon the ventricle during its diastole as well as during its systole.

Ludwig and Luchsinger,[1] in their experiments upon the entire heart, appear to have varied venous pressure alone.

A cannula was put into the vena cava inferior, connected with a pressure bottle, and the aortic arches cut through. Pressure was varied by means of the pressure bottle. In this case pressure was exerted upon the interior of the heart during both systole and diastole, differing from true arterial pressure, which acts directly upon the heart only during ventricular systole.

They found that increase of pressure caused an increase of pulse rate.

It was the object of our experiments to leave the entire heart in position in the body, cut off all external nervous influences, and then, keeping up a constant venous pressure by means of a Marriott's flask, to vary the arterial pressure alone.

Our method of operating with the terrapin, which we have used in most cases, was to remove the plastron, slit open the pericardium, bind the small ligament running from the ventricle to the pericardium, the two superior cavæ, the left hepatic vein, the pulmonary artery, and put cannulas into the right and left aortas, (this was done merely in case one should clot); one aorta, usually the right, was connected with the manometer and outflow tube during an observation, while the other was clamped. Finally a cannula was put into the inferior cava and connected with the Marriott's flasks. The animal's heart was washed free from all coagulable blood, the vagi and sympathetics cut, the latter below the middle cervical ganglion, the head cut off, and the cervical spinal cord destroyed. The heart was then allowed to run from half an hour to an hour before any observations were made.

Essentially the same method was used with the frog; the arterial cannula was put into one of the aortic arches before its external division into three trunks.

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[1] Ludwig and Luchsinger. Zur Physiologie des Herzens. **Pflüger's Archiv,** June, 1881.

We, at first, tried to feed the hearts with salt solution 0.6 per cent., but found that the beat soon became weakened too much to give a pulse in the manometer. Defibrinated calf's blood, filtered through linen, and diluted with an equal bulk of 0.6 per cent. salt solution was then tried; it was found to work admirably. We have kept the heart under experiment four or five hours, and it was just as good at the end of that time as at the beginning; it was kept moist by lying in a small pool of the blood poured into the visceral cavity of the animal.

Apprehending some trouble in the use of a mercury manometer (which did not occur, however), we endeavored to make a water manometer. We tried, at first, the one mentioned in the June number of Pflüger's Archiv, 1881, by Gruenhagen, but found that it would not do for our purpose, since the paraffin stem floated so little above the level of the water, that practically no variations of pressure could be registered with it. We then, with the aid of Dr. Sewall, devised a water manometer which worked very satisfactorily. The manometer we used (*M*, Pl. XVI) is made of glass tubing having an internal diameter of 7 or 8 mm., the limb in which the float works is about 40 cm. long, the other about 6 cm. The whole of the interior of the manometer is coated with a thin layer of paraffin. For a float, *S*, we use a very light glass stem, made by drawing out a thin test tube; this is also coated with a layer of paraffin, and has a small bulb, *b*, blown on the end which is immersed in the water. A small cork float, *f*, well soaked in paraffin, with a diameter a little less than the internal diameter of the manometer, has a hole bored through its centre, and is then slipped down the glass stem, so as just to touch the surface of the water, when the stem is allowed to float freely in it. If the stem sinks too low in the water, or is unsteady, one or more of these little paraffined cork floats may be placed on that part immersed in the water.

The top of the stem has a light glass pen fastened to it with sealing wax, and can be made to write upon a drum.

The manometer is provided with a glass cap, the opening through which the stem works being well paraffined.

The stem in our manometer is about 38 cm. long, and sinks in the water 17 cm., allowing us to register variations of pressure of about 20 cm. of water. It is difficult to get a stem longer than this that is not bent so much as to make it useless.

The float follows very accurately every motion of the water, and gives excellent tracings.

We used besides this a small mercury manometer having an internal diameter of about 1.75 mm.

Plate XVI represents the apparatus used by us in our experiments.

A and B are the Marriott's flasks, and are used alternately. H is the heart, represented as separated from the body, though such was not actually the case. a is a piece of stiff rubber tubing leading from the aorta; at C there is a three-way tube, one branch of which passes to the manometer, while the other (O) serves as an outflow tube for the blood pumped out of the heart. By raising or lowering this tube any desired arterial pressure can be obtained. By means of a screw clamp on O we were also able to change arterial pressure, to block the outflow entirely, or to alter the height of the pulse wave. With very low arterial pressure, for instance, it was very often found necessary to diminish considerably the lumen of the outflow tube, in order to get a distinct pulse wave in the manometer.

A pressure bottle, not represented in the drawing, was used to fill the manometer and its connections.

Tracings were taken upon an ordinary revolving drum, upon which wrote also a chronograph pen marking seconds.

In our later experiments before isolating the heart, we took the blood pressure of the animal used, filling the cannula for this purpose with 0.6 per cent. salt solution, or defibrinated calf's blood. In the terrapin this pressure was taken in the left aorta, in the frog in one of the aortic arches.

As the general result of our experiments, we can state that *variation of arterial pressure, up to the highest point of normal blood pressure, has no direct effect whatever upon the pulse rate of the isolated frog or terrapin heart.*

In the terrapin we could carry the arterial pressure to more than twice the normal blood pressure, without affecting the pulse rate. Excessive pressure, however, caused in most cases a slight slowing of the pulse, the slowing varying as a rule from 2.5 per cent. to 9 per cent. of the normal pulse rate, in some cases more.

In the frog arterial pressure could not be carried much above the normal without causing a slight slowing due to secondary

influences: very high aortic pressure may so distend the aorta as to make the semilunar valves insufficient to close it: or may be so great as to prevent the ventricle from carrying out a proper contraction and maintaining the circulation. We are carrying out further experiments with reference to these points; the latter of which is probably the more important. With high pressure little, and with the outflow tube completely blocked, no renewal of the blood takes place in the heart, and Luciani found, that when the serum in an excised frog's heart is renewed, the pulse becomes more frequent.

The following tables give some of the results obtained. As a general thing observations were made at intervals of five minutes, of which two were taken up by the revolution of the drum; the pressure would then be raised or lowered, as the case might be, to the next desired height, and the heart allowed to work at that pressure for about three minutes, before another tracing was taken. The pressure and rate of heart beat remained remarkably constant for any one revolution of the drum. The tracings were divided up into sections of twenty seconds each, and the average beat per minute deduced from these.

Pressure was measured from a base line taken at the end of the observation. In the tables " venous pressure " indicates the pressure at which blood was supplied from the Marriott's flask to the vena cava. The temperatures given are those of the room. The blood supplied to the heart, and the animal experimented upon, were always kept in the room a considerable time before commencing an experiment. As will be seen, we could not always keep the temperature constant during an experiment; and this had sometimes a marked influence on the rate of beat of the heart.

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Table I.

	Time. P. M.	Temperature in degrees C.	Average Pressure in cm. of water.	Average Beat per minute.
November 30. Terrapin cura-	4.20	21 5	12	35.5
rized. Head cut off at the	4.25	21.5	32	35
second or third cervical	4.30	21.5	12	35
vertebra. Vagi and sym-	4.35	21.5	31	35
pathetics cut, and cervical				
spinal cord destroyed. Ve-	5.45	24	12	39.25
nous pressure = 2.1 cm.	5.50	24	31.5	39
Water manometer used.	5.55	24	11.5	38.5
	6.00	24	31.5	38.5
	6.35	22	12	37
	6.40	21.5	22	37
	6.45	22	32	37
	6.50	22	32	37
	6.55	22	22	37
	7.00	22	12	37
	7.25	22	12	36
	7.30	22	22	36
	7.35	22	32	36.33
	7.40	22	32	36
	7.45	22	22	36
	7.50	22	12	36
	8.05	22	32	36.4
	8.30	22	32	36
	8.40	22	12	36
	8.50	22	22	36

Table II.

	Time. P. M.	Temperature in degrees C.	Average Pressure in mm. of mercury.	Average Beat per minute.
December 1. Terrapin curarized. Head cut off at the second or third cervical vertebra. Vagi and sympathetics cut, and cervical spinal cord destroyed. Venous pressure = 4 cm. Mercury manometer used.	4.05	23	3	36.1
	4.10	22.5	16.5	35.6
	4.15	22.5	30	35.4
	4.30	23	34	35.5
	4.45	23	2	36.4
	4.50	23	34	37.5
	4.55	23	3	37.5
	5.00	23	34	37.7
	6 20	23	2	40.8
	6.25	23	13	40.5
	6.30	23	33	40.5
	6.35	23	33	40.3
	6.40	23	15	39.3
	6.45	23	1	39
	7.00	22.5	1	37.8
	7.05	22.5	21	37.5
	7.10	22.5	42	37.5
	7.15	22.5	2	37
	7.20	22.5	15.5	37.5
	7.25	22.5	40	37.5

Table III.

	Time. P. M.	Temperature in degrees C.	Pressure in cm. of water.	Average Beat per minute.
December 8. Large Frog. Brain and spinal cord destroyed. Both water and mercury manometer used. Venous pressure, during the first part of the experiment, = 3 cm.	1.55	21	14	49
	2.00	21	34	49.25
	2.05	21	14	48
	2.10	21	33.5	48
	2.24	21	14	49.25
	2.29	21	24	48.75
	2.35	21	34	48.5
	2.40	21	14	48.8
	2.50	21	34	48.25

Table III.—*Continued.*

	Time. P. M.	Temperature in degrees C.	Average Pressure in mm. of mercury.	Average Beat per minute.
Venous pressure, mercury manometer used, = 1.5 cm. In this case it was noticed that with pressure above 29 mm. of mercury the ventricle	3.55	21	2.5	42
	4.00	21	31.5	38.5
	4.10	21	2.5	39.5
	4.15	21	28	39.75
was never emptied, indicating a partial giving away of the semilunar valves, or that the tension in the aorta was too great for the ventricle to overcome.	4.30	20.5	3.5	40.25
	4.35	20.5	10.5	40.5
	4.40	20.5	19	40
	4.45	20.5	26	39.25
	4.50	20.5	33.5	37
	5 00	20.5	5	40.5
	5.05	20.5	13	41.25
	5.10	20.5	23	41.25
	5.15	20.5	28.5	40.5
	5.20	20.5	33	37.75

Table IV.

	Time. P. M.	Temperature in degrees C.	Average Aortic Pressure in mm. of mercury.	Average Beat per minute.
December 10. Terrapin. Blood pressure in left aorta before commencing the observation = 20 mm. Hg. Vagi and sympathetics cut, head cut off and cervical spinal cord destroyed. Venous pressure = 2.6 cm. Mercury manometer used.	3.40	21	3.5	31.25
	3.45	21	10	32 85
	3.50	21	20	33.18
	3 55	21	27.5	33.45
	4.00	21	36	32.4
	4.05	21	43	31.86
	4.10	21	43	31.86
	4.15	21	37.25	32.81
	4.20	21	32.75	33.45
	4.25	21	27	34.5
	4.30	21	14.5	33.72
	4.35	21	4	33.75

Table IV.—*Continued.*

	Time. P. M.	Temperature in degrees C.	Average Aortic Pressure in mm. of mercury.	Average Beat per minute.
The rise in pulse rate that took place towards the end of this series is probably due to the rise of temperature in the room. It is clearly independent of the pressure in the aorta.	4.45	21	4.5	34.68
	4.50	21.5	12.7	34
	4.55	21.5	25.5	34.5
	5.00	21.5	33.1	84.5
	5.05	22	38	84.5
	5.10	22	42	34.5
	5.20	22.5	44	34.5
	5.25	22.5	37.1	34.7
	5.30	22.5	35	34.8
	5.35	22.5	29	35.3
	5.40	23	17	35.4
	5.45	23	5	36
	5.55	23	5	36
	6.00	23	22.5	36
In this case an aortic pressure nearly three times that found before the observations commenced (57 to 20) was obtained by completely blocking the outflow tube—the heart pumping into the manometer only. The heart was kept in this condition about ten minutes—from 6.03 to 6.13. The very abnormal pressure slowed the heart and the slowing effect remained some time after the heart was relieved.	6.05	23	57	33.37
	6.10	23	53.75	29 62
	6.15	23	22.25	34.69
	6.20	23	5	35.81

	Time. P. M.	Temperature in degrees C.	Pressure in cm. of water.	Average Beat per minute.
Water manometer put on. Same venous pressure.	6.50	22.5	11	42
	6.53	22.5	20	42.25
	7.00	22.5	30	42

Table V.

	Time. P. M.	Temperature in degrees C.	Average Pressure in mm. of mercury.	Average Beat per minute.
December 13. T e r r a p i n. Blood pressure taken from left aorta before isolating heart = 16 mm. Vagi and sympathetics then cut, head cut off and cervical spinal cord destroyed. Venous pressure = 4.5 cm. Mercury manometer used. The rise of temperature at the end of the series caused a slight quickening of the pulse.	7.30	23	2	43.1
	7.35	23	9.5	42.8
	7.40	24.5	20 5	43.5
	7.45	24.5	27.5	42.1
	7.50	25	33	44.25
	7.55	24.5	38.5	44

Table VI.

	Time. P. M.	Temperature in degrees C.	Average Pressure in mm. of mercury.	Average Beat per minute.
December 14. Frog. Brain and spinal cord destroyed. Venous pressure = 4.5 cm. Mercury manometer used.	2.00	23	4.75	55.5
	2.05	23	12	55.5
	2.10	23	20	55.5
	2.15	23	26.5	55.5
	2.20	23	33	54
	2.25	23	37	52.6
	3.10	23.5	4	57.7
	3.15	23.5	12	57.7
	3.20	23.5	25.25	57
	3.25	23.5	32	56.4
	3.30	23.5	35.5	55.5
	3.35	23.5	37	55.5
	3.40	23.5	34	56.4

Table VI.—*Continued.*

	Time, P. M.	Temperature in Degrees C.	Average Pressure in mm. of mercury.	Average Beat per minute.
	3.45	23.5	28.5	57
	3.50	23.5	19	57.6
	3.55	23.5	4.9	58
	4.10	23.5	3.75	57
	4.15	23.5	14	56.7
	4.20	23.5	27	57
	4.25	23.5	33.6	56.4
	4.30	23.5	37	55.6
	4.35	23.5	35.25	56
	4.40	23.5	30.2	57
	4.45	23.5	23	57
	4.50	23.5	14	57
	4.55	23.5	3	57

Our thanks are due to Professor Martin, for advice and suggestions during the course of the work, which we think shews conclusively that within wide limits variations in aortic pressure do not in the least influence the rate of beat in the heart in the animals experimented upon.

SOME NOTES ON THE DEVELOPMENT OF ARBACIA PUNCTULATA, Lam. By H. GARMAN and B. P. COLTON. With Plates XVII and XVIII.

IT was the privilege of the writers to spend some time last season at the marine laboratory of Johns Hopkins University at Beaufort, N. C., and while there to make some observations on the development of *Arbacia punctulata* which seem of sufficient interest to warrant publication. The development, from the first changes after fertilization of the ova to the formation of the young sea-urchin and the resorption of the pluteus, was under constant observation. Materials were thus accumulated for a complete history of the development, as far as external changes are concerned, but since the earlier stages do not differ essentially from those of other Echini, and have been fairly well figured and described by Dr. J. W. Fewkes,[1] we shall not at present give more than a few notes on some of the later stages of the pluteus and on the young sea-urchins, thus supplementing, in some measure, the work already done. Our thanks are due to Dr. Brooks, director of the laboratory, for facilities afforded us in pursuing the work, and for other assistance.

Arbacia punctulata appears to be the commonest sea-urchin at Beaufort. Great numbers of them were brought up in the trawl from the deeper water of Bogue Sound opposite Morehead City. They were also taken in some numbers about the piers of wharves at low tide. *Strongylocentrotus dröbachiensis* was represented by frequent examples of the form with white spines. *Mellita testudinata* was the only other echinoid at all common. The handsome

[1] Mem. Peab. Acad. of Science, Vol. I, No. VI, 1881. In this memoir Dr. Fewkes figured and described most of changes in the developing Arbacia pluteus, but did not follow the development to the appearance of the young sea-urchin, of which we were fortunate enough to rear a number of specimens and would doubtless have obtained more could we have stayed longer at Beaufort. The Figure 20 of Dr. Fewkes' plate is unlike any Arbacia pluteus we have seen. While our plutei varied within certain limits and were sometimes deformed, in the many specimens examined we saw none that had more than two pairs of arms on the oral lobe where Dr. Fewkes represents three.

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bleached shell of this sand dollar was a common object on the shoals. A single example of a fourth species was taken by Mr. Rice.

The eggs of Arbacia were readily fertilized artificially. The ovaries and testicles with ripe contents were taken from the living animals, placed in a watch-glass containing sea-water and cut into bits with a pair of scissors. The watch-glass was then emptied into a beaker full of sea-water and the contents of the latter gently stirred with a glass rod. Here they remained until the pluteus emerged, an event which took place about six hours after the fertilization of the ova. Portions of the water containing plutei were then poured into a number of beakers of fresh sea-water, leaving the undeveloped eggs and remnants of the ovaries and testes in the bottom of the first vessel. By this means the plutei were given more room and materials likely to render the water impure were got rid of. Afterwards, as the plutei grew, individuals were dipped up from time to time with tubes and transferred to separate glasses. It was found best to filter the water used, thus removing creatures likely to prey upon the young plutei. The vessels were usually kept covered to prevent dust and insects from falling upon the water.

Ova and spermatozoa could be obtained from Arbacia at any time while we were at Beaufort (from the middle of July till the latter part of September), but after the first of September difficulty was experienced in fertilizing the eggs. Many lots were tried, but in most cases after one or two divisions of the egg-contents, the development became abnormal and soon ceased altogether. The spawning period seemed to have passed, and the reproductive organs, though still with apparently ripe contents, were much less distended than earlier in the season.

The development of the egg takes place, under favorable circumstances, with great rapidity. In one instance the first segmentation was noticed just twenty-five minutes after fertilization, and at times divisions of the egg-contents took place within fifteen minutes of each other. In another lot the first division was not noted until an hour and a half after the eggs were fertilized, and the periods between divisions varied to a similar extent. The eggs from which the plutei were obtained upon which most of our work was done, were fertilized on the 16th of August, at 9 o'clock, A. M., and at 3.40, P. M., of the same day the first plutei had

emerged. The plutei obtained from these eggs were under observation till the 22d of September, at which time a number of young sea-urchins had emerged.

In these notes we follow the majority of authors in calling that surface of the body on which the vent opens, ventral, and the opposite one dorsal. The anterior part of the body is that in which the mouth opens. The right and left sides will then be those seen to right and left respectively when the pluteus lies with the dorsal surface up and the mouth-lobe from the observer. This explanation seems necessary to prevent misapprehension, as Dr. Fewkes calls the surface in which the vent opens, dorsal.

The living plutei usually remained near the surface of the water, the constantly moving cilia apparently serving chiefly as means of maintaining the equilibrium. The anterior part of the body is almost invariably carried uppermost, as one would expect from the form of the body, the long, slender arms projecting upwards, while the posterior portion being more compact, tends to sink lowest. The movements of the perfect Arbacia pluteus are not rapid. It is not built for speed, and when it moves forwards, does so with the widest end foremost. But while the most rapid movement cannot be effected in this way, the main object of movement is attained most admirably. The large mouth and movable lip are held in a position to collect the particles of food brought into contact with the broad, concave front, and the animal thus travels as a sort of self-acting surface net. The younger plutei move more actively, though less steadily. The peculiar form causes them to turn as they move, first on one then on the other side.

The matured pluteus is semi-transparent and is marked with red pigment spots. This pigment is very abundant towards the tips of the arms. It is also disposed in spots along the calcareous rods of the arms, and occurs as patches and dots on other parts of the body. The oral lobe bears two pairs of arms. The longer pair (Fig. 1, 2) arises from the dorsal surface at the sides of the lobe and extends forwards. The arms of the shorter pair (Fig. 1, 5) project obliquely downwards and forwards from the sides of the anterior border of the lip. The lip (Fig. 1, *b*) is a large, freely movable flap which partly covers the mouth in front, and at times entirely closes it. The mouth (Fig 1, *a*) opens beneath the lip, is very large, and gapes wide open. It opens into a large muscular œsophagus (Fig. 1, *c*), by the peristaltic contractions of which food

is carried to the stomach (Fig. 1, *d*). Previous to the formation
of the young sea-urchin, the stomach occupies the greater part of
the body-cavity. The opening from the œsophagus into it usually
remains closed while that from the stomach into the intestine
stands open. The intestine (Fig. 1, *e*) arises from the posterior
under part of the stomach, extends forwards, and opens on the
ventral surface a short distance behind the anterior border of what
may be called the ventral lobe. Two pairs of very long arms
arise, one (Fig. 1, 1) from the anterior lateral angles of the ventral
lobe, the other (Fig. 1, 4) from the sides of the oral lobe about
opposite the first pair. Both pairs project forwards, the dorsal
obliquely upwards and the ventral obliquely downwards. The
remaining pair of arms (Fig. 1, 3) arises from what is termed the
anal lobe, and the arms project obliquely outwards and slightly
backwards. There is a thickening of the central portion of the
anterior border of the ventral lobe which continues backward on
each side and forms the margin of a pair of ciliated "epaulets"
(Fig. 1, *i*). A similar pair of epaulets (Fig. 2, *j*) occurs on the
dorsal surface. They are supported anteriorly by prongs of
the skeleton. The size of the plutei and the relative length
of the arms is subject to considerable variation. The variation
in the arms does not appear to be due so much to resorption as
to a symmetrical development. The resorption of the pluteus
takes place chiefly, as will be seen later, within a short space of
time after the sea-urchin is protruded. This asymmetry may have
been due to the unnatural conditions in which the plutei were
living, and probably in nature little of such variation occurs.

Deformities were frequent; one pluteus observed was club-
shaped, consisting of little else than a stomach and single arm.

The calcareous skeleton prevents any range of movement of the
arms, but in the later stages of development a frequent drawing
apart and closing of the long lateral arms may be observed, and
in some instances the posterior pair was seen to move back and
forth. The union of the rods of the arms of this pair prevents
independent movement, so that when one moves forwards the
other moves to the rear. The arms appear in the following order:
First, the arms of the ventral lobe (Fig. 1, 1); second, the longer
pair of arms at the extremity of the oral lobe (Fig. 1, 2); third,
the arms of the anal lobe (Fig. 1, 3); fourth, the pair from the
sides of the oral lobe (Fig. 1, 4); and fifth, and last, the small

pair on the anterior border of the lip (Fig. 1, 5). The arms, lip, and membranous folds are supplied with cilia. The separate parts of the skeleton appear as minute spicules. The first spicules to appear are four-rayed. They develop near the ventral surface just behind the anterior border. The lowest ray develops rapidly and pushes down into the anal lobe, where it unites a little later with a corresponding ray from the spicule of the opposite side. The lateral ray pushes across the body near the ventral surface and finally unites at the middle line with its fellow from the opposite side. A third ray grows towards the dorsal surface, and curving forwards, grows into the second pair of arms as they develop. The fourth ray supports the long first pair of arms.

A crescent-shaped spicule next appears at the posterior end of the stomach near the points of origin of the third pair of arms, and sends a branch from near its extremities into each arm. The third spicules are triradiate and appear with the fourth pair of arms. One ray of each spicule supports an arm, and the remaining rays project, one obliquely forwards and the other obliquely backwards.

The only other spicule appears just above the œsophagus in the middle line. The two lateral rays curve outwards and forwards and support the fifth pair of arms. A small prong develops from each of these lateral rays for the support of the anterior projection of the dorsal fold (Fig. 2, *k*). The third ray does not develop. The transverse bar formed by the union of rays from the first spicules is, later in development, broken by the resorption of its material at the point of union. The long rods of the first pair of arms also unite at an early stage within the anal lobe, to be again broken towards the close of pluteus life by resorption. Still another change occurs. The rods which support the second pair of arms originally form a part of the first spicules, but become freed later by resorption. The meaning of these changes becomes clear when we consider the sudden metamorphosis which closes the pluteus stage. During the earlier periods of its existence there is need of a strong support for the fragile body, but as the last change approaches, greater freedom of movement is called for and can be secured only by breaking the unions of the rods. The larger rods of the skeleton are perforated by series of oval or round openings and are usually more or less spinose. Figure 3 shows the skeleton at a stage represented by Figure 1.

The rods are numbered to correspond with the arms they support. The prong for the support of the dorsal fold has not yet appeared.

The young sea-urchins were first noted when the pluteus was about two weeks old. At this stage the larvæ rest on the bottom, swimming but little. On the left side of the stomach the tube-feet appear (Fig. 2, *h*). There are five of them in a circle extending outwards from the abactinal disc which rests upon the side of the stomach, their free ends approaching each other and forming a cone. In a dorsal or ventral view usually only two or three can be seen, but towards the left side the five are shown presenting a radiate appearance. Over the apex of the cone, *i. e.* over the approximated free ends of the tube-feet is the opening to the exterior. Already the growth of the young sea-urchins presses on the stomach, flattening it, and later, pushes it towards the opposite side so that it occupies the smaller part of the width of the body. The tube-feet early show contractility. Around the circle of tube-feet is a circle of flattish lobes, the beginning of the first developed marginal spines. As the tube-feet develop, they are from time to time protruded slightly through the opening, being thrust out farther and remaining out longer as growth proceeds. By the turning of the lateral arms outwards and backwards the opening is enlarged and the feet pushed out. At first the feet only, later the spines at their basis appear outside. Figures 4 to 7 show the young sea-urchins in various degrees of exertion as seen from different points. In the complete act of everting the lateral arms turn back passing astride the apical pair swinging outwards like the ribs of an umbrella turning inside out. At the same time the oral lobe is drawn to one side. Then the arms return to their former position, the feet are withdrawn, the opening is almost entirely closed and the appearance is again as in Figure 1. Specimens were observed to repeat this process about once an hour for hours in succession, remaining everted a quarter or half an hour. When extended the feet are moved about and by applying the discs to the surface on which the pluteus rests, effect a slight degree of locomotion ; but the movements are awkward, the long projecting arms making the pluteus top-heavy. This process is kept up for days and in some cases for weeks, the eversions becoming more and more complete until finally the everted state becomes the permanent one, the three principal pairs of arms extending from what

was the apical end of the body, and the oral lobe is drawn out of its original position as in Figure 7. Soon the rods pierce through the ends of the arms and the softer tissues of the arms slide down the rods and are withdrawn into the body. The bare rods are left projecting. In endeavoring to crawl, the newly emerged sea-urchin frequently topples over and some of the rods are broken off; all of them soon disappear. The oral lobe also soon disappears. The complete absorption of the arms and oral lobe occurs within a very short time.

The young sea-urchin is semi-transparent and marked with pigment spots similar to those of the pluteus. They are especially abundant on the peristome. The shell is strong. About the margin of the corona is a series of fifteen spatulate spines. From above the five large tube-feet may be seen separating the spines into sets of threes. On the actinal surface about the mouth many small tube-feet are placed. The developing Aristotle's lantern with its muscles and ligaments can be made out. The structure is apparantly the same as in the adult. The plates of the periproct are now relatively very large. A little later the five large tube-feet are reduced in size, and above them appear five additional spines. They eventually become longer than the original fifteen, are narrower and more pointed. The radioles are articulated to the corona as in the adult, and are so attached as to slope downwards. At first they are serrate distally, but the serratures soon disappear. The large tube-feet have a perforated calcareous plate in the disc, and in the smaller feet the forming plate appears as minute spicules. Large pedicellariæ soon appear on the abactinal surface, and the corona becomes studded with scattered tubercles. Figures 8 and 9 represent the young sea-urchin soon after the resorption of the pluteus.

The young sea-urchin aids its movements to a considerable extent by pushing downward and laterally with its flattish spines, thus early showing the use of the spines in locomotion which is characteristic of the adult. The spines can be bent down at right angles to the plane of the body, but can be bent upward but very little, owing to the projecting rim at the base of the spine above the obliquely inserted pedicel. At this stage the young sea-urchins were frequently observed climbing up the sides of the glass vessels in which they were kept.

EXPLANATION OF THE FIGURES.

PLATE XVII.

FIGURE 1.—Ventral surface of the Arbacia pluteus. *a*, mouth; *b*, lip; *c*, œsophagus; *d*, stomach; *e*, intestine; *f*, anus; *g*, opening of stomach into the intestine; *h*, first appearance of spines and tube-feet of the young sea-urchin; *i*, the ventral epaulets. The arms, 1–8, are numbered in the order of their development.

FIGURE 2.—Dorsal view of pluteus at a little later stage of development than the preceding. Signification of letters and figures the same. *j*, dorsal epaulets; *k*, spine-like rod supporting the anterior part of the epaulets.

FIGURE 3.—Dorsal view of the calcareous skeleton. 1, rods supporting the first pair of arms; 1*a*, prong which at an earlier stage was united with the corresponding one of the opposite side; 2, rods supporting second pair of arms and at one time united with 1; 3, support of the third pair of arms; 4, supports of fourth pair; 5, spicule supporting the fifth pair of arms and sending up branches for the dorsal epaulets.

FIGURE 4.—Appearance of the pluteus when almost ready to transform. On the left side (right in the figure) is seen the invagination through which part of the tube-feet and spines of the sea-urchin appear. The stomach is pushed to the right.

PLATE XVIII.

FIGURE 5.—The pluteus with the tube-feet and part of the spines protruded, as seen obliquely from the front. The lateral arms are turned partly backwards and appear on each side of the third pair. The oral lobe with the first and fifth pairs of arms (1 and 5) are drawn to one side.

FIGURE 6.—Side view, showing the feet and spines still more protruded, with the lateral arms turned completely back, having passed astride the third pair.

FIGURE 7.—Transformation nearly complete. The pluteus undergoing resorption. *l*, the remains of the oral lobe.

FIGURE 8.—Ventral view of the young sea-urchin, as seen on the twenty-fourth day after the fertilization of the ova. *a*, the five large tube-feet; *b*, the smaller, later-formed tube-feet; *c*, marginal spines of the corona.

FIGURE 9.—Abactinal surface of an older individual, showing the five additional radioles and the first pedicellariæ.

Figures 2, 5, 6, 7 and 8 were drawn by Mr. Colton. Figures 1, 3, 4 and 9 by Mr. Garman. With the exception of Figures 3 and 9 all were drawn from the living plutei.

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ON THE STRUCTURE AND SIGNIFICANCE OF SOME ABERRANT FORMS OF LAMELLI-BRANCHIATE GILLS.

By K. MITSUKURI, Ph. B., of Tokio, Japan, Fellow of the Johns Hopkins University, Baltimore. With Plate XIX.

THE following contribution to the morphology of the Molluscan branchiæ is part of an investigation on which I have for some time past been engaged, under the direction of Dr. W. K. Brooks, in Professor Martin's laboratory at the Johns Hopkins University. The gills, of which the description is here given, are those of *Nucula proxima* and *Yoldia limatula*. They are extremely interesting because of their simple structure, and this account of their minute structure is published with the hope that it might throw some additional light on the nature of Lamellibranchiate gills. I wish to express here my sincere thanks to Dr. Brooks for his constant advice and assistance. I am also deeply indebted for specimens used in the investigation to Professors A. E. Verrill and S. J. Smith, of Yale College, and to Mr. Richard Rathbone, of the United States Fish Commission.

Nucula proxima, Say.

This Lamellibranch shows many departures from the structure which is generally regarded as characteristic of the class. Figure 1 gives a fair idea of what is seen when the left valve of the shell has been taken away, and the mantle of the same side removed along the lower border of the visceral mass near the line *x y*. *a. a.* is the anterior adductor muscle made up of several fasciculi; *p. a.* is the posterior adductor. It will be noticed that *Nucula* possesses one of the few shells in which the umbo is turned toward the posterior end. In the specimen figured, the visceral mass (*v. m.*) shows convolutions on the surface, which, under the microscope, proved to be the male reproductive organ, probably enormously developed for the breeding season, and this character enables one to distinguish

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the sex of a specimen without difficulty. All the males have these convolutions, and, when preserved in alcohol, are of a greyish color. The females show hardly any convolutions, and are much more darkly colored. The foot (f) is folded longitudinally at its end, and can accordingly be spread out into a flat circular disc. The labial palpi (l) are unusually developed, and might at first sight be taken for gills. The inside of the outer and the outside of the inner palpus are raised into numerous parallel ridges, which, as shown in the figure, can be seen from the outside, and do not extend to the lower margin. At their posterior end there are two remarkable structures. One of them is a hood-like structure ($l. b.$, Figs. 1 and 2), which is the posterior prolongation of the united upper edges of the inner and outer palpi. The other ($l. a.$, Figs. 1 and 2), lying immediately below the first, is a long tentacular appendage. It is a hollow tube, open, however, along a line on its posterior aspect, and having its cavity continuous with the space between the two palpi. As it has been seen protruded, with the foot outside of the shell (Woodward's "Manual of Mollusca," p. 426), and since, in alcoholic specimens, a great deal of dirt and sand is found along its length and between the palpi from its base to the mouth, it is no doubt a food-procuring organ, probably sending a constant stream of nutritive matters to the mouth by means of its cilia. It is interesting to notice in connection with this appendage that in *Nucula*, the gills, unlike those of ordinary Lamellibranchs, must be practically useless for obtaining food, as will be evident from the following description of them.

The gill ($g.$, Figs. 1 and 3) is comparatively small. It is situated quite posteriorly, and is suspended by a membrane ($m.$, Figs. 1 and 3), which is attached to the body along the broken line $x\,y\,z\,w$. It is united to the visceral mass ($v. m.$) from x to y, and to the upper part of the foot (f, Fig. 3) from y to z (see Figs. 1 and 3). At the last point (having come to the median line of the body) it joins with its fellow of the opposite side, and they continue in this way as far as w. Here they separate again, each proceeding to the posterior tip (p) of the gill of its own side. It should be remarked that, as the point x is further from the median line of the body than the point y (Fig. 3), there is a considerable free space beneath the suspending membrane of the gill.

When we turn to the gill itself, we find an altogether unusual structure. Figure 4 shows it dissected out and seen from below

and slightly from one side. In general appearance it resembles a
boat which is suspended by its keel. $x c p$, Figure 4 (seen in cross
section at $i j$, Fig. 5), is the line of attachment and corresponds to
the keel; $x d p$, Figure 4 (seen in cross-section at d, Fig. 5), rep-
resents the bottom line of the hollow of the boat. The latter is
bounded by the two surfaces $x a p d$ and $x b p d$ (Fig. 4; seen in
cross-section at $b d$ and $a d$, Fig. 5). The anterior end (x, Fig. 4)
is rather blunt, while the posterior end p, Figs. 1, 3, and 4)
is quite pointed. The resemblance of the gill to a boat is, however,
only very superficial, as the gill is not one solid mass, but is made
up of a series of paired plates of a peculiar shape, placed one after
another from the anterior to the posterior end. A little dissection
under a lens will show that the part above the line $x d p$ (Fig. 4)
and below the line of suspension ($x c p$), is continuous along the
entire length of the gill, and that, with this part for the *stem*, the
plates are given off, one after another, in pairs to the two sides
(see Fig. 5). The plates constitute the proper respiratory parts of
the organ. They are largest in the middle, and diminish in size
toward the two extremities.

It is evident from this description that the gill in *Nucula* is of
quite an exceptional nature. It does not, as in most Lamelli-
branchs, extend along the whole length of the side of the body,
constituting the most conspicuous object of the mantle cavity, but
is comparatively insignificant, being pushed back and freely sus-
pended in the mantle cavity. It cannot, therefore, divide the
latter into the suprabranchial and infrabranchial chambers, and is,
of course, utterly devoid of any structure like the ciliated water-
passages in the ordinary gill, for driving water from the lower to
the upper. It cannot, also, as has been said, serve as an effective
food-procuring organ. The gill in *Nucula* must for these reasons
be of vastly less functional importance to the animal than it is in
common Lamellibranchs, and, so far as I am able to see, serves
only as the organ of respiration. It seems to me, however, that
the division of the mantle cavity into the upper and lower cham-
bers is begun in the posterior part. It has been seen that ventral
to the membrane suspending the gill (m, Figs. 1 and 3) there is a
large space continuous with the general branchial cavity, and there
certainly is a space dorsal to this membrane. These spaces seem
to be the rudiments of the supra- and infrabranchial chambers.
Moreover, the arrangement of the different parts at the posterior

end, as seen in Figure 3, recalls that of the corresponding parts in many of those genera in which the mantle cavity is divided into two parts. It is not difficult to conceive how the same division might be brought about in the case of *Nucula*, by proper development of the gill and the membrane.

Figure 5 shows a pair of opposed plates considerably enlarged. The solid part (*i d j*) which I have called the *stem*, and which is continuous throughout the whole length of the gill, together with the suspending membrane (*k i j l*) is seen in cross-section in the middle, and from this middle portion the paired plates (*e. e.*) are seen to proceed. The colored part at the bottom represents the complex chitinous framework. The membrane (*k i j l*) is made up of fibrous tissue, the bundles of which this is composed crossing each other in many directions. Its free surfaces are covered with columnar epithelium. The stem consists mostly of a solid mass of large irregular cells with rather large nuclei. There are, I am almost certain, *two* blood-channels excavated through it; a lower larger (*n*), and an upper smaller (*o*). The latter seems to be in connection with a free space (*q*) found often in sections of the suspending membrane. The large channel (*n*) sends a branch (*r*) into each plate. The fibrous tissue found in the upper membrane dips down into this part at regular intervals, viz: between every branch (*r*) of the lower blood-channel (*n*). How these fibres end below, when they reach the chitinous framework, I have not been able to make out. A few fibres (*u*) are sent down into the plate a little above the blood-channel (*r*), and gradually approach and finally touch the latter near its lower end. A few more fibres (?) are seen along the upper edge of the plate. Exactly what this fibrous tissue is I am unable to make out, but it seems to be some sort of tough connective tissue, with perhaps muscular fibres more or less intermixed. That it is very tough and serves as a support to the whole structure is seen by the fact that the fibres often stick out beyond the broken edge of the soft tissue. The trough of the chitinous framework is seen at *s*, in cross-section. It extends along the whole length of the gill and sends out two branches into each plate. I have obtained the appearances, in some sections, of a bundle of fibrous tissue running in it and filling it. The framework will be described more fully further on. The plates (*e*), the proper respiratory organs, are comparatively speaking very broad and quite thin, and hang down from the solid part of the gill.

The epithelium of the plates which is represented in the figure as ending abruptly at the edges *i d* and *j d*, turns at a right angle at these lines to cover the *stem*, and is soon reflected outwards again to form the epithelium of the next plate in the series. This is evident from an inspection of Figure 8. Each plate may be said to be simply an enormously widened blood-channel (Fig. 6), and as the blood is necessarily spread out in a thin layer over a large area, the purposes of aëration must be admirably served. The columnar epithelial cells seen at *a d*, Figure 5, are very characteristic of the plates under a microscope, and are the cells (*d a*, Fig. 6) around the chitinous bars (*h*, Figs. 5 and 6) seen in optical section. The surface of the irregularly rectangular cells placed just inside these columnar cells in Figure 5, ought therefore to be continuous with the outer edge of the columnar cells, but in order to avoid confusion is not so represented in the figure. This is also the case with the cubical cells along the upper edge. The chitinous support (*h*, Figs. 5 and 6) of the plate runs near the lower edge (Fig. 5) to its tip (*a* or *b*, Fig. 5), and is made up of two entirely separate parts (seen in cross-section in Fig. 6) applied closely together. Owing to the shape of these parts there is, however, a narrow oval space between them. This space, as will be shown further on, is continuous with the space in the trough (*s*, Fig. 5) of the *stem*. The cells along the lower edge of the plate are columnar, and surround the chitinous support in a characteristic manner shown in Figure 6. Their surface outlines are irregularly rectangular, contrasting with the irregularly polygonal cells covering the rest of the plate. The branch (*r*, Figs. 5 and 6) of the lower blood-channel (*n*) in the *stem*, is seen to be circular in cross-section and to bulge out the surface of the plate. These points are not, however, constant, as the vessel is sometimes constricted into more or less separate channels, while the amount of bulging seems to depend on the quantity of blood present. The remaining part of the plate (*e*, Figs. 5 and 6) is flat and quite thin, enclosing a broad blood-channel between its two epithelial surfaces. It is here no doubt that the aëration of blood is accomplished. The cells of this part are cubical, as seen in Figure 6. Some of them send processes inward to join others from the opposite side. This gives a labyrinthine appearance to this part of the plate. The course of the blood is evidently from one blood-channel in the *stem* to the other, through the space in the plate.

For instance, the blood may start from the upper channel (*o*) in the stem, go to the broad flat part (*e*, Fig. 5) of the plate where it gets aërated, then enter the branch (*r*), along its upper edge, and run up this to reach the lower blood-channel (*v e*) in the stem. This is, however, a purely hypothetical course. I have had no means of determining whether the blood goes from the upper to the lower channel or *vice versâ.*

The framework which supports the gill can be separated out by heating it in dilute caustic potash, as it is insoluble in weak acids and alkalies. It is stained by carmine and other coloring reagents. Whether it is really formed of chitin I do not know, but as previous writers have described the substance as of that nature it will be convenient to use the term "chitinous support" for the present. The framework consists of a trough (seen in cross-section at *s*, Fig. 5; longitudinally from below in Fig. 8; diagramatically represented in Fig. 7) which runs along the whole length of the gill, and from which a pair of closely-applied parallel branches (*h*, Figs. 5, 6, 7 and 8) is given off into each plate. The trough is divided into two unequal parts: an upper larger and a lower smaller, by a cross piece (*c p*, Figs. 5 and 7), which stretches from one side of it to the other, a little below the middle. This cross piece is not, however, continuous, but is pierced through by oral openings (*o v*, Figs. 7 and 8) whenever branches are given off laterally into the plates. The space enclosed between each pair of closely-applied branches (see *h*, Figs. 6, 7 and 8) is connected with the lower compartment of the trough by means of somewhat circular openings (*o p*, and *o' p'*, Figs. 7 and 8) found near the bottom. In Figure 8 the letters *a, a, a*, are placed opposite each pair of the branches that go into a plate. It will be seen how one-half of the chitinous support of one plate, after forming an arch at the trough, turns round to enter the next plate in succession, and to constitute there one-half of the support of that plate. The framework treated with potash, and sometimes without any treatment, shows marked longitudinal striation (Fig. 8), and some of its fibres sticking out at the broken edge beyond the others resemble in appearance the fibres found in the suspending membrane, at *t* and *u*, Figure 5, and give reasons for thinking that the whole chitinous framework is nothing but the fibrous tissue found in other parts cemented closely together and forming one cohering mass.

Although, owing to the state of the specimens, I have obtained only here and there evidences of cilia, it seems reasonable to suppose that the whole gill is covered with cilia. On two rows of cells (*l. f.*, Fig. 6 ; *d. a.*, Fig. 5) on the lower edge of the plate I believe there are larger cilia than on the rest, as I have now and then seen their remains, and as, without any question, cells in the corresponding positions in *Yoldia* have long and conspicuous cilia.

Yoldia limatula, Say.

Yoldia resembles *Nucula* in several structural peculiarities—in its well-developed labial palpi, with their peculiar food-procuring appendage, in its feather-like gills, in the posterior position and comparatively small size of the gills, and the consequent absence of the division of the mantle cavity into the supra- and infra-branchial chambers. It differs from *Nucula* in having a siphon, and further shows its departure from the ordinary lamellibranchiate structure in having a highly specialised tactile organ in the siphon.[1]

The gill, although different in details from that of *Nucula*, is essentially of the same structure as the latter. It is suspended by a membrane, as in *Nucula*. Figure 9 shows it dissected out by itself. The line of suspension is *x c p ; x d p* is the ventral median line, and corresponds to *x d p* in Figure 4. As in *Nucula*, the gill is made up of a series of paired plates, placed one after another, and attached to the central solid *stem* continuous throughout the whole length of the gill. The plates do not, however, project downward, as we have seen in the case of *Nucula*, but here turn upward (see Fig. 11). The plates are largest in the middle, and gradually become smaller toward the extremities. At the front end (*x*, Fig. 9) there is a rather interesting arrangement. Figure 10 shows diagramatically the relations of the various parts at the anterior termination of the gill. It will be seen that the plates of the gill gradually become smaller and finally die out toward the front, and the gill is continued simply as a flat membraneous structure (*x*, Fig. 10), which goes into the visceral mass (*v. m.*, Figs. 9 and 10). A cross-section of this part shows that at its lower portion at least, there is a blood-channel, probably continuous with one

[1] W. K. Brooks. Proc. Amer. Ass. Adv. Sci., 1874 (end of note).
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of the channels in the *stem* of the gill. In some specimens this membrane-like portion of the branchia is longer than in others, and goes some distance around the visceral mass.

Owing to the rather poor state of preservation of the alcoholic specimens, I have not been able to make out the histology of the *Yoldia* gill as fully as I should like, but the following description I believe to be correct in essential points :—Figure 11 represents an opposed pair of plates, and corresponds to Figure 5 of the *Nucula* branchia. The suspending membrane ($k\ i\ j\ l$) consists of fibres crossing each other in several directions, and is covered on its two surfaces by columnar epithelium. The solid *stem* ($i\ d\ j$) of the gill has two blood-channels, an upper (n) and a lower (o). The latter seems to be in communication with a comparatively free space (q) in the middle of the suspending membrane. Directly below the upper blood-channel (o) there is a bundle of tissue, which appears to be fibrous, running the length of the gill (seen in cross-section at f, Fig. 11). It serves no doubt for support. The floor of the lower blood-channel (r) is covered by a ᐯ-shaped bundle of longitudinal fibres (s). This would seem to be homologous with the trough-shaped chitinous structure in *Nucula*, but seems to be formed of the same fibres already referred to several times, which are found in the suspending membrane and other parts of the *Nucula* and *Yoldia* gills, and I cannot establish any connection between this bundle and the chitinous bars (h, Figure 11) in each plate. The latter, when they reach the longitudinal bundle (s), make a bend and turn out again to enter the next plate in the series. In some sections I have obtained indications of a very thin layer of chitin beneath the fibrous bundle (s), which may, therefore, correspond to the fibres found *in* the trough of the framework in the *Nucula* gill (see above). If, however, this ᐯ-shaped structure is really homologous with the *trough* of the *Nucula* gill, it goes far in support of the view advanced above, that the chitinous framework is really made up of the fibrous tissue which is found in other parts, here cemented into one compact mass. In such a case fusion has gone further in *Nucula* than in *Yoldia*, and we see in the first genus the trough well united with the branches (h) in each plate. The plates (e, Fig. 11) in *Yoldia* spread themselves upward instead of downward, as in *Nucula*. The chitinous bars (h), of which there are two in each plate, follow the curve of the plate and end rather bluntly about

half-way up, at the point *a*. That the part from *d* to *a* corresponds to the lower inner edge of the *Nucula* plate (*d a*, Fig. 5) is shown by the characteristic rows of columnar cells having longer cilia than those found in other parts of the gill. There is another system of chitinous structures (*ch*, Fig. 11). Many fine chitinous filaments come down together in a bundle on each side from the suspending membrane, and as soon as each bundle reaches the plate of its own side filaments spread themselves out like the frame of a fan over the whole plate. Several fibres sometimes proceed together, and then separating, give the appearance of branching. They are found directly beneath the epithelial cells that cover the plate. The effect of this framework must be to keep the plate well spread out for the purpose of aëration. I have not succeeded in obtaining any single section which shows the structure of the plate well, but from the comparison of a good many sections which I have made, I feel tolerably sure that the whole space between the epithelial surfaces is pervaded by what Peck[1] calls "lacunar tissue" (Fig. 12). It is a loose trabecular tissue with many nuclei and within whose network blood can flow. The space between the chitinous bars (*h*, Fig. 11), which is quite large in *Yoldia*, seems to be tolerably free from this lacunar tissue. Figure 11, *a*, gives the outline of the plate seen from one side.

Theoretical Considerations.

The gills, here described, of *Nucula* and *Yoldia* are, I think, the most rudimentary of any that have been studied so far. In fact, at first sight, the resemblance to the ordinary Lamellibranch gill is not apparent, and they suggest more the Cephalopod gill. But I believe, the homology of their various parts with those of more complex gills in *Unio, Mytilus, Arca*, &c., is not difficult to make out. After consulting the articles by Peck (*loc. cit.*), Posner,[2] Lacaze-Duthiers,[3] Bonnet,[4] and others, and also after examining

[1] R. Holman Peck. "The Minute Structure of the Gills of the Lamellibranch Mollusca." Quar. Journ. Micros. Sci., 1877.

[2] Carl Posner. "Ueber den Bau der Najadenkieme." Archiv für mikros. Anat., 1875.

[3] Henri de Lucaze-Duthiers. "Mémoire sur le Developpement des Branchies des Mollusques Acephales Lamellibranches." Ann. d. Sci. Nat., Ser. IV, Tome V, 1856.

[4] Robert Bonnet. "Der Bau u. die Circulations-Verhältnisse der Acephalenkieme." Morphologisches Jahrbuch, III, 1879.

the sections I myself have obtained of *Unio, Modiola, Scapharca,*
&c., I have no doubt whatever that the *plates* in *Nucula* and
Yoldia represent the descending or attached limb of the filaments
in the *outer* and *inner* gill-plates in forms like *Mytilus, Modiola,*
and *Arca,* and accordingly are homologous with the folds on the
inner lamella of the *outer* gill-plate, and on the *outer* lamella of
the *inner* gill-plate in *Unio, Anodon,* and *Dreissena.* If a com-
parison is made of my Figure 6 with any of the cross-sections of
gill-filaments given by Peck, it will be seen at once how similarly
the paired chitinous bars are placed, how almost identically the
epithelial cells are arranged around them, how two rows of those
cells (*l. f.,* Fig. 6)—called by Peck latero-frontal epithelial—have
longer cilia than the rest.   In fact, Peck's Figure 12 (a transverse
section of a filament of the Anodon gill) agrees with my Figure 6
in all essential points.   The left-hand figure in his Figure 5 (the
superficial view of the edge of a gill-filament of *Mytilus* showing
the latero-frontal and other epithelial cells) and the upper part of
his Figure 20 (the same view of a gill-filament of Anodon) would
pass very well for the corresponding part in *Nucula.*   So far as I
can make out from rather poor specimens, the latero-frontal cells
in *Nucula* are strikingly like those represented in Peck's Figure
20.   If, then, the plates in the gills of *Nucula* and *Yoldia* repre-
sent the gill-filaments in other genera, it follows from the embryo-
logical observations of Lacaze-Duthiers (*loc. cit.*), and from the
position of the chitinous bars in the plates, that they are homolo-
gous with the *descending* limb of the gill-filaments in ordinary
Lamellibranchs.   Professor Huxley seems to have no doubt what-
ever of the homology stated here, as will appear from the quota-
tion given further on.   Admitting, then, that this supposition is
correct, and that the gills in *Nucula* and *Yoldia* are in an unusually
rudimentary condition, what light, if any, do they throw on the
organogeny of the Lamellibranchiate gill?   But, before proceed-
ing to the discussion of this point, let us review briefly what
theories have been advanced as to what is the most primitive type
of the branchiæ of this group.   Setting aside older authors like
Williams and Hancock, I consider the articles, already alluded to,
by Peck, Posner and Lacaze-Duthiers as having the most impor-
tant bearing on the subject.   Posner, after a careful histological
examination of the gills of *Anodon, Unio, Cardium, Mya, Mytilus,*
Ostrea, Pecten, Pholas, Pinna, Scrobicularia, Solen, Solecurtus, and

Venus, puts forward, although with hesitation, the theory that the pouch-like gills of the Unionidæ are the most primitive type of the Lamellibranchiate gill. Stepanoff,[1] so far as I can gather, inclines to this view. Peck, on the other hand, after an investigation of *Area*, *Mytilus*, *Anodon*, and *Dreissena*, comes to the conclusion that "the gill-plates of the Unionidæ are a highly modified form derived from a simple condition in which the gills consist *not* of plates but of a series of juxtaposed independent *filaments*, such as we see in a less modified state in Arca and Mytilus." This view is the more generally accepted of the two. The only complete history of the development of the Lamellibranchiate gill by Lacaze-Duthiers (*loc. cit.*) and all the fragmentary embryological observations on the organs show that the gills are at first of a tentacular or filamentary character. Those who read carefully Mr. Peck's paper, will, I think, feel convinced by the arguments he brings forward. So high an authority as Professor Huxley is entirely of this view. He says: "In its simplest form, the branchia of a Lamellibranch consists of a stem fringed by a double series of filaments (*e. g. Nucula*). The next degree of complication arises from these filaments becoming, as it were, doubled upon themselves at the free ends, the reflected portions lying on the outer side of the outer, and on the inner side of the inner, series of filaments . . . (Mytilus Pecten). In most Lamellibranchs, the gills are four elongated plates, each of which is in fact a long narrow pouch, with its open end turned toward the hæmal face of the body " (*Invertebrates*, p. 408–9, Am. Ed.). My own observations lead me to the same conclusion. In fact, it is difficult to see how the pouch-like gills of *Unio* can give rise to such forms of branchiæ as are found in *Nucula* and *Yoldia*. By a very circuitous route they may have degenerated into their present rudimentary state, it is true, but all recent observations tend to show that while other organs in the Lamellibranchiata have been steadily degenerating, the gills, on the contrary, have become highly developed and perform functions which the probable change of the animal from the motile to the sedentary habits of life has forced on these gills. If, then, there has been no considerable degeneration, and if the homologies of different parts of

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[1] **Paul Stepanoff.** "Ueber die Geschlechtsorgane und die Entwicklung von Cyclas." Archiv f. Naturgesch., 1865.

these branchiæ are, as I have stated above, the filamentary char-
acter of the primitive Lamellibranchiate gill is placed beyond
doubt.

I believe further light is thrown on the subject by the gills of
Nucula and *Yoldia*. Peck shows that the gills primarily consisted
of a series of filaments, but does not attempt to account for the
fact that these filaments have come out in long rows on the side of
the body. I venture to suggest an explanation. If we reflect for
a moment, I think we shall see that the gills of *Nucula* and *Yoldia*
may be considered as a stem which, being folded on either side to
increase the surface of contact with the water, gives rise to the flat
plates which I have homologized with the descending limb of the
gill-filament of *Mytilus* and other like forms. The plates are,
strictly speaking, nothing but the epithelial covering of the stem
raised into folds and enclosing between the two sides of the folds
a blood-channel. In the case of *Yoldia* mesoblastic lacunar tissue
is carried out into the folds. According to this theory, the gill of
the Lamellibranchiata was originally a longitudinal ridge on the
side of the body. Probably in this a blood-vessel ran, and must
have served as the organ of respiration. In course of time, how-
ever, this ridge became folded for the increase of the surface of
contact with the water and thus produced papilla on its two
sides—rudiments of the future gill-filaments. The gills of *Nucula*
and *Yoldia* have gone but little beyond this stage. I think there
is much to support this view. Stepanoff (*loc. cit.*) observed in
Cyclas that the gills arise first as a ridge on each side of the
body. Leydig[1] makes the same statement. M. Lovén's[2] observa-
tions have a still more important bearing on the point. He says:
"Nous avons, si je ne me trompe, vu la première formation des
branchies; nous en savons assez pour être sûr qu'elles se montrent
sous la forme d'un cordon fin, renflé à certains intervalles; que ces
renflements se contournent plus tard en anses, qui s'allongent de
plus en plus, et sur lesquelles se développement les cils vibratiles

[1] Franz Leydig. "Ueber *Cyclas cornea*" Müller's Archiv, 1855. He says:
"Die letzte Hauptänderung im äusseren Habitus erfährt der Embryo durch
die Bildung der Kiemen. Auch sie wachsen als *Leisten* von hinten nach vorne
und zwar gehen sie ursprünglich vom Mantel aus" (p. 62).

[2] "Bidrag till Kämedornen om utvecklingen af mollusca acephala Lamelli-
branchiata." Memoirs of the Academy of Stockholm, 1848, lately reprinted
in an abridged form in German.

regulièrement disposés et d'un forme particulière." [1] " Un cordon fin renflé à certains intervalles " is, it seems to me, nothing but a ridge with slight swellings or papillæ. Lovén's figures are not exactly clear to me, but what he designates as the gills are certainly in favor of my view. In all the fragmentary embryological observations, the gills are generally seen as papillæ, or nothing but the folds of a blood-channel. I have already called attention to the anterior part of the *Yoldia* gill where the plates die out and the gill is continued simply as a ridge containing a blood-channel. Whether this is a remnant of the primitive ridge or not it is difficult to determine, but the fact that there *can be* on the side of the body a thin-walled ridge which, containing a blood-channel, must serve more or less for respiration, goes far in support of the view here advanced.

To review the whole matter, the Lamellibranch gill was perhaps originally a simple ridge on the side of the body, but to increase the surface of contact with the water folds may have arisen on two sides of this ridge. If such was the case, *Nucula* and *Yoldia* are still in a stage only very little advanced from this primitive condition. In course of time, however, as some of the Lamellibranchiata, either owing to degeneration or some other cause, become incapable of extensive locomotion, these buds or folds were perhaps prolonged to form tentacular filaments, which, going on in their development, finally produced such complete gill structures as we see in *Mytilus, Unio, Ostrea,* and other forms, taking on at the same time functions totally foreign to their original one. Between the simple gills of *Nucula* and most complex ones known, there are a great many intermediate stages, some going more in one direction, others in another. For instance, *Lucina* and *Corbis* are said to have only one gill-plate on each side (*Owen's Inverteb.*). According to Sars, *Pecchiola* is in the same condition (*Remarkable Forms of Animal Life,* G. O. Sars). *Chamostrea* and *Myochama* are described by Hancock (*Ann. and Mag. of Nat. Hist.,* 1852–3) as having the inner gill-plate complete, but the outer plate lacking the outer lamella. In these tentacular filaments seem to be fused with each other. On the other hand, although *Arca, Mytilus, Modiola,* have all the lamellæ present, the filaments composing

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[1] Translated by M. Young and quoted by Lacaze-Duthiers in the article already referred to.

them have not fused with one another. It is interesting to notice that *Nucula* and *Yoldia*, in which the gills have remained rudimentary, have, as Dr. Brooks first pointed out to me, an unusual power of locomotion, while forms wholly or almost wholly unable to move, as *Ostrea*, *Pholas*, &c., possess highly-developed gills.

For some reason the inner gill-plate seems to develop further than the outer. For instance, in many genera, the inner is much larger than the outer. In *Chamostrea* and *Myochama*, already referred to, it is the inner gill-plate that is complete, and the outer gill-plate that lacks a lamella. It will also be seen a little further on that in *Anodon* the inner gill-plate has gone further than the outer in its development. In the embryological study of the branchiæ of *Mytilus*, Lacaze-Duthiers observed that the filaments of the inner gill budded out first.

It is very instructive to see the process of secondary folding going on in higher varieties of the gill. The two lamellæ of a gill-plate are, in such a case, no longer parallel, but wavy, and the surface of a lamella is thus considerably increased. In *Anodon* this process is perhaps going on, for Peck shows that in that genus the cross-section of the outer gill-plate has parallel and straight edges, but that the outer lamella of the inner has a wavy margin. Posner shows successive stages of secondary folding in the gills of *Pholas dactylus*, *Venus* (sp.), *Mya arenaria*, *Ostrea edulis*, *Solen vagina*, *Cardium edule*, *Pinna nobilis*.

Diametrically opposite, as the views advocated by Posner and Peck may seem, it is not difficult to reconcile the two.

If we look over the list of the genera examined by Posner, we shall find all of them, except *Mytilus* and perhaps *Pecten*, to possess more complex gills than *Unio*, and starting, as he did, from the last genus, it is no wonder that he considered it to possess the primitive gill. On the other hand, Peck investigated forms simpler than *Unio*, and arrived at the probably true conclusion. Posner simply began where Peck ended. The two investigators, therefore, supplement each other, and now, with the addition of the extremely simple gills of *Nucula* and *Yoldia*, the series is fairly complete, and it seems to me that the filamentary character of the primitive Lamellibranch gill is made tolerably certain.

OBSERVATIONS ON THE EARLY DEVELOP-MENTAL STAGES OF SOME POLYCHÆTOUS ANNELIDES.

By EDMUND B. WILSON, Ph. D., *Assistant in Biology, Johns Hopkins University.* With Plates XX, XXI, XXII and XXIII.

In the course of two seasons' work at the Chesapeake Zoölogical Laboratory, I made a few observations on the earlier stages of development in a small number of marine Annelides. While these observations are in many respects superficial and incomplete, they nevertheless concern precisely those stages of development which are least known and in regard to which current ideas appear to be somewhat erroneous. I am therefore led to publish a brief account of my observations, if only for the sake of affording a basis for more thorough future study.

Although a number of writers have contributed to our knowledge of the segmentation of the eggs of Polychæta, I have seen no satisfactory account of the early stages of that process in those forms characterized by an unequal segmentation. Most of the statements in regard to it, as pointed out in the sequel, appear to be somewhat erroneous and give no hint of the close similarity which exists between the segmenting Polychætous egg and those of many other animals (*e. g.,* some *Oligochæta, Hirudinea, Dendrocœla,* Pulmonate Gasteropods). Furthermore, the later stages of the segmentation are of some interest, since the separation of the germ layers is the result of a process which appears to be, in some respects, intermediate between an epibolic invagination and a kind of irregular or progressive delamination. In one or two Polychætous Annelides, as we know from Stossich's and Giard's observations, the segmentation is nearly or quite equal, a large segmentation cavity appears, and a gastrula is formed by embolic invagination. In vastly the greater number of cases, however, the segmentation is decidedly unequal, and a more or less modified epibolic gastrula results. The passage from this mode of development to a modified form of delamination is not hard to imagine, and it appears to be actually exemplified, to some extent, in the

development of *Clymenella, Arenicola* and *Chætopterus*, as described farther on.

The young stages of American Annelides are very imperfectly known. Mr. Agassiz's valuable and well-known observations stand almost alone, though there are a few scattered notes by other writers. My observations relate to five genera, viz: *Clymenella, Arenicola, Chætopterus, Spiochætopterus,* and *Diopatra. Chætopterus* is confined, so far as I know, to the region south of the Chesapeake; the others have a much wider range. It will be convenient to describe these forms in the above order.

Clymenella torquata (Leidy), Verrill.

As in many other cases (*e. g., Terebella, Protula, Dasychone, Spio*) the eggs are inclosed in a semi-fluid gelatinous substance which forms in this species an ovoid mass about the size and shape of a pigeon's egg. At one extremity the mass suddenly narrows to form a peduncle which passes into the mouth of the tube inhabited by the worm. These egg-masses were found at Beaufort, N. C., from May until late in September, and in such abundance as to form a very characteristic feature of the beach and shoals. They extend from half-tide down to a depth of two or three fathoms, and are consequently exposed to the air for several hours each day; the embryos appear to sustain this exposure without injury. The egg-mass contains several hundred eggs, which are ovoid bodies measuring, on the average, about .21 mm. in length and .16 mm. in diameter. The vitellus, which is surrounded by a very distinct chorion, is rendered very opaque by the presence of a large quantity of granular food-material or dentoplasm. For this reason I have not been able to study the behavior of the segmentation nuclei and other internal phenomena of the segmentation; my observations relate, therefore, almost solely to the external changes.

I was unable to determine when the eggs are fertilized, but think it probable that this process takes place before the eggs are laid, which is certainly the case with some other Annelides. No direction cells are formed—at any rate, none which occupy any definite position with respect to the vitellus.

Segmentation begins with the division of the vitellus into two unequal parts (Fig. 2), after which the two spherules thus formed

become pressed together and a period of quiescence ensues; this continues twenty or thirty minutes. The spherules then assume a more rounded form and are soon divided into four parts (Fig. 3) by a furrow passing nearly at right angles to the first. This division takes place in such a plane as to divide the smaller of the two primary spherules into two equal parts (*b, c*, Figs. 4, 5), while the larger primary spherule is divided into unequal parts (*a, d*), of which the larger (*a*) is at the left side (the egg being viewed from the upper or micromere pole). The four spherules soon become flattened and closely pressed together, and a second resting-stage ensues (Fig. 6). After a quiescence of about twenty minutes the spherules again swell up (Fig. 4) and four smaller spherules are separated from them by a horizontal furrow, passing in a plane at right angles to the two preceding (Figs. 7, 8, 9). The four spherules thus formed, being smaller, may be called micromeres, and the four larger ones macromeres. The micromeres are not always produced at the same moment (Fig. 7), but the difference in time is very slight. The substance of the micromeres does not differ in appearance from that of the macromeres, being still very opaque from the presence of granular food-material. No difference in in this respect between the macromeres and micromeres can be seen until a much later period, although it is from analogy very probable that the micromeres contain from the first a greater proportion of protoplasm. As shown in Figure 8, each micromere lies, at first, directly above the macromere from which it has separated. In a short time, however, each micromere moves to one side so as to come opposite the interval between two macromeres, the spherules become closely wedged together and the egg passes into a third resting-stage of about the same duration with the two preceding. This shifting of the micromeres is a common occurrence in the similar eggs of other animals (*e. g., Clepsine, Bonellia*); it appears to be a result of the mutual attraction of the spherules since they are thereby enabled to fit more closely together. It is worth noting that one of the micromeres is invariably a little larger than the others; this is the one derived from the spherule marked *d* in Figure 4—that is, the smaller of the two unequal spherules resulting from the division of the primary larger spherule (see Fig. 2).

After the formation of the first four micromeres we are enabled to determine the relation of the parts of the embryo to those of

the adult worm. The micromeres occupy the dorsal side, the macromeres the ventral; and the largest macromere marks the posterior end. The mouth is formed, long afterwards, at a point nearly opposite to the micromeres.

The following figures are for the sake of convenient comparison placed with the posterior end below. With the close of the third resting-stage the segmentation loses its regular rhythmical character, and the spherules henceforth multiply independently of each other, undergoing in their development, though less conspicuously, alternations of activity and quiescence like those which have hitherto been passed through by the entire egg. Thus it comes about that while one part of the embryo is showing signs of rapid change, another part may be almost stationary.

After the third resting-stage renewed activity is begun with the division of the micromeres. Figures 12 to 17 represent the progressive changes of an egg. The lower or posterior micromere was first to divide (Fig. 13); this was followed, two minutes later, by the right-hand micromere (Fig. 14) and, after another minute, by the left-hand micromere (Fig. 15). The upper micromere did not divide, apparently, until considerably later. Five minutes after the division of the last micromere the left-hand macromere was divided (Fig. 16) into an upper smaller part, in all respects like the micromeres, and a larger part lying below and at the side of the egg (*cf.*, Fig. 18). Soon afterwards the large posterior spherule divided, a smaller spherule separating from its left side. The latter soon divided again into a smaller upper part (*a*, Fig. 17) and a larger lower part (*b*).

Examining the opposite side of an embryo at this stage, we find (Fig. 18) five or six spherules considerably larger than those on the other side. The spherule *ab* appears to have separated from the large posterior macromere and to have produced the spherule *a* of the preceding figure. (For a detailed study of the changes of the lower pole the reader is referred to the account of the development of *Arenicola*, p. 279).

Figure 19 represents the upper side of an embryo thirty minutes later. The micromeres now form a layer of somewhat uniform cells over the top of the embryo. If the egg is examined from the lower pole the macromeres are found to have multiplied also, though they are still considerably larger than the micromeres. But at the sides of the embryo are spherules of intermediate size,

and no dividing line between macromeres and micromeres can be drawn. The only point where a definite limit to the layer of micromeres can be assigned is at the posterior end where they adjoin the large posterior macromere. The latter may sometimes be observed in the process of division (Fig. 20) and it appears to bud off smaller spherules which become incorporated into the layer of peripheral cells.

Figure 21 is a somewhat oblique side-view of an embryo ninety minutes later. To the left are cells resulting from the division of the micromeres, and these appear to graduate into the larger cells to the right, which are derivatives of the macromeres.

Figures 22 and 23 represent ventral and dorsal views of an embryo of a somewhat later stage. As before, the micromeres are distinctly smaller than the macromeres, but the two kinds of cells graduate into each other at the sides of the embryo. The posterior macromere is still very large; dorsally it appears to be overlaid, to some extent, by the micromeres.

At about this stage, or sometimes a little earlier (Fig. 24), the large macromere divides into two (Figs. 25, 26). A side-view at about this stage (Fig. 27) shows, as before, the micromeres passing gradually into the macromeres, some of the latter near the posterior end being in the course of active multiplication. From this time the multiplication of the macromeres (if they can still be so called) appears to be somewhat accelerated, so that the peripheral (*i. e.*, ectodermic) cells become more nearly of equal size. The two large macromeres are gradually lost to view, being in part, as I believe, overgrown by the ectoderm and in part used up to supply smaller peripheral cells. Cells may often be observed in active division at this part of the embryo while the other cells of the ectoderm are quiescent.

At about this period (14 hours, Fig. 29) the anterior part of the embryo becomes less opaque, and in some specimens the large polygonal cells of the entoderm may be seen. The entoderm cells, it is important to note, are distinctly larger than any of the peripheral cells, though the latter are as yet larger upon the ventral than upon the dorsal side. Posteriorly, the limit between ectoderm and entoderm is invisible. A section of the embryo at about this stage (Fig. 28, *a*) shows the entoderm to consist of a solid mass of very granular cells, the limits of which are ill defined in the section. In the anterior and middle regions of the egg the entoderm is

definitely separated from the ectoderm, the latter being clearer
but with only obscure indications of the cell walls. Behind the
middle, however, this definite limit disappears, and we find large
cells with conspicuous nuclei which form no definite layer and are
continuous with the granular entoderm cells within. The ecto-
derm layer seems to abut against these large cells and not to overlie
them. It appears to me highly probable that the further back-
ward and downward extension of the ectoderm is, to some extent
at least, produced by the separation of the outer ends of the large
cells as ectoderm cells, and furthermore that this process takes
place not only in the later but also in the earlier stages of develop-
ment. The spherule marked a in the figure is apparently under-
going such a division, but the section does not show this definitely
enough for certainty. It appears to be generally the case with
epibolic invagination that the micromeres receive, for a time at
least, constant additions from the macromeres, and the extension
of the ectoderm is due to this process as well as to the multiplica-
tion of the primary micromeres. So long as the two kinds of
spherules are of very unequal size and differ perceptibly in consti-
tution, the layer of micromeres can be seen to grow around and
envelop the macromeres. But if, as in the present case, the micro-
meres and macromeres differ little in size from the time of
their first appearance, the separation of a micromere from a
macromere must be the division of one of the larger spherules into
an outer ectodermic cell and an inner entodermic one, which is, so
far as it goes, a process of delamination. This process, however,
takes place progressively from above downwards and backwards,
so that the last parts of the ectoderm to be formed are those at
the posterior extremity of the embryo, where the anus, at a much
later period, is formed. While this appears to be the general
nature of the process in *Clymenella*, it is quite possible that the
four primary micromeres contain no entodermic part, and that a
part at least of the large posterior micromeres are at last actually
overgrown by the advance of the ectoderm without contributing
cells to that layer. Further discussion of this mode of develop-
ment is deferred until after a description of the segmentation of
Arenicola and *Chætopterus*.

The embryo gradually elongates, and when about 24 to 30 hours
old (Fig. 31) acquires a broad band of short cilia surrounding the
anterior part; this is soon followed by a second much narrower

band (Fig. 32) near the posterior end. The ventral surface also becomes uniformly ciliated except upon a narrow interval in front of the posterior ring. The chorion has remained, during all these stages, and now forms a very distinct cuticle which is perforated by the cilia. This cuticle persists in later stages and from the outermost layer of the body of the young worm.

The larvæ now swim slowly through the mass of jelly, rotating slowly about the longitudinal axis. In favorable specimens (Fig. 33) the large entoderm cells are visible and the layers are sharply separated at the anterior extremity. Posteriorly, however, and in the region of the ciliated bands, the ectoderm is very opaque and cannot be clearly distinguished from the entoderm. Figure 1, Plate XXIII, represents the larva of sixty hours, at which age it sometimes leaves the jelly and swims for a time slowly about in the water. More commonly, however, it remains in the egg-mass during a much longer period.

The growth of the larva to the adult takes place in the usual manner by elongation of the body and the continual formation of somites in regular succession from the posterior region. The segmentation is at first expressed externally only in the arrangement of the setæ; it is only in late stages that the external lines of division between the somites become visible. About the third or fourth day a pair of eye-specks appears just in front of the anterior ciliated band. Figure 2, Plate XXIII, represents the larva five days old, taken from the egg-mass. Four setigerous somites have appeared, but the larval cilia remain as before, though they no longer extend to the apex of the præoral lobe and are disappearing from the ventral side behind. The mouth is now visible and lies behind the anterior ciliated belt. The setæ number two or three in each somite; they are all setiform and belong to the dorsal ramus, no uncini having as yet appeared.

The young *Clymenellas* lived more than a month in the aquarium, when they had acquired fifteen setigerous somites and some of the characteristic external features of the adult. The anal funnel is developed from a series of rounded papillæ surrounding the anal opening. The uncini (setæ of the lower ramus) are not developed until the setæ of the upper ramus have appeared in a number of segments. Like the latter, the uncini first appear in the anterior segments, but the order of their development is less regular than that of the upper setæ.

I have not observed how the mouth and anus are formed. At the close of segmentation no blastopore is visible, and the mouth appears much later on the ventral side in or behind the anterior ciliated belt. The anus appears to arise still later at the posterior extremity where the blastopore, if present, should by analogy be found. The alimentary cavity is hollowed out in the middle of the entodermic mass long before any communication with the exterior can be found.

In the oldest larva observed the proboscis is well developed and is protrusible; the last remnants of the anterior ciliated band still persist in front of the mouth, and the larval eyes are still present; there is still a single uncinus only in the anterior somite.

Arenicola cristata, Stimpson.

The segmentation of the eggs of this species is so similar to that of *Clymenella* that it will be unnecessary to give so detailed a description of it.

The eggs are embedded in huge gelatinous masses which assume various forms as they are swayed to and fro by the tide. A common form is irregularly cylindrical, three or feet long and as many inches in diameter. Sometimes they are rounded and shapeless, lying flat on the sand; in other cases they are as long as six feet or more and from one to three inches in diameter. The size of these masses is enormous, considering the dimensions of the adult worm, and this is the more striking from the fact that the egg-masses of *A. marina* (*piscatorum*, Auct.) as described by Max Schultze[1] are hundreds of times smaller, being scarcely a fourth as large as those of *Clymenella*, and containing only three or four hundred eggs. The number of eggs, in the case of our species, must reach several hundred thousand. They are small (the average diameter being about .13 mm.), nearly spherical or slightly oval in form, very opaque, and are inclosed in a remarkably thick chorion which, seen by oblique light, appears to be perforated by minute radiating pores. The vitellus is of a light cinnamon color, so that the egg-mass appears of a decided reddish brown tint.

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[1] Abhandlungen der naturforschenden Gesellschaft zu Halle, 1855, printed in 1856.

The early stages of development resemble those of *Clymenella* so closely that one set of figures might almost answer for both. No direction-cells were observed.

The vitellus first divides into two unequal parts which soon flatten together somewhat, and a resting-stage of about twenty minutes ensues (Figs. 35, 36, 37). The second cleavage (Figs. 38, 39) takes place exactly as in *Clymenella* and is succeeded by a second resting-stage (Fig. 47). The third cleavage takes place in the horizontal plane, separating four micromeres from the upper pole of the egg (Figs. 40, 49). As in *Clymenella*, the micromeres become shifted so as to lie between the macromeres instead of over them, and the egg passes into a third resting-stage (Figs. 41, 50). As before, one of the micromeres is a little larger than the others. It is a noteworthy fact that the micromeres, as compared with the macromeres, are distinctly larger than in the *Clymenella* egg; that is to say, the segmentation is less unequal. ·

After a quiescence of fifteen or twenty minutes activity is resumed; we will first follow the changes at the lower or macromere pole. The macromeres divided, in the specimen figured, almost simultaneously (Fig. 42), each giving rise to a smaller anterior and a larger posterior spherule (these are connected in the figure by short lines to show their derivation). Very soon afterward the micromeres also divide (Fig. 52, from another egg), and the egg passes into another pretty marked resting-stage (Fig. 43) of about twenty minutes duration. From the large posterior macromere a smaller spherule then separates (Fig. 44) on the right side, and the other macromeres divide in somewhat irregular succession (Figs. 44 to 46).

Figures 51 to 53 represent the changes (in another egg) of the upper pole. The micromeres form a cap of smaller cells which are behind clearly separated from the macromeres, but elsewhere graduate into the latter. Owing to the great opacity of the eggs, I have not been able to follow the subsequent changes as fully as in *Clymenella*, but so far as could be determined, they were essentially similar to those of the latter. Two or three spherules at the posterior end retain for a long time their predominance in size (Fig. 54) and appear to be in part overgrown by the peripheral cells. Elsewhere, the micromeres and macromeres graduate into each other, and the macromeres appear to separate, in the course of their development, into peripheral parts which become incorporated

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into the ectoderm and central parts which pass into the entoderm. Figures 55 and 56 are opposite views of an embryo about nine hours old. The blank space at the bottom of Figure 56 is the posterior extremity; the cell outlines could not definitely be made out in the specimen.

The subsequent external development differs from that of *Clymenella* only in matters of small detail. The embryo gradually elongates, and when 18 to 24 hours old (Fig. 57), acquires a broad anterior belt of cilia, in front of which appear two eye-specks. Very soon a second belt, much narrower than the first, appears near the posterior extremity, and the ventral surface becomes covered with a broad band of short cilia, which, however, does not extend quite to the posterior ring (Fig. 58). The first pair of setæ appear during the third day (Fig. 59). The head is now distinct and the mouth has appeared on the ventral side, apparently in the middle of the ciliated belt; at a later stage it lies behind this belt. The chorion of the earlier stages now forms a very distinct transparent cuticle which is perforated by the cilia; this cuticle persists in the latest stage observed.

At about this stage the larvæ leave the egg-mass and swim about actively at the surface of the water. They always swim towards the lightest side of the vessel, where they crowd together in such numbers as to form a cinnamon-colored scum on the water. The free-swimming life is very brief, lasting commonly no more than a day or two. Figure 3, Plate XXIII, represents a larva of four days which was slowly swimming about near the bottom of the vessel. The body now shows an obscure segmentation, and a new pair of setæ has appeared behind the former pair. A new seta has also appeared on each side in the anterior setigerous somite. In front of the two setigerous somites is a segment of the body without setæ, and in front of this is the head. By the fifth day another somite with a pair of rudimentary setæ is developed from the posterior region, and a second pair of setæ appears in the next somite in front. These setæ all belong to the upper ramus; the new ones appear below the older ones, so that the setæ develop from above downwards. In a few specimens of this age an uncinate seta of the lower ramus has appeared in the anterior setigerous somite. The cilia have begun to disappear, and though many of the larvæ are still swarming at the surface, they secrete a gelatinous substance which greatly impedes their move-

ments. They soon, however, sink to the bottom, or attach themselves to the sides of the vessel. Here they secrete small masses of a soft gelatinous substance in which they creep actively about. Thus conditioned, they lived more than three weeks in the aquarium. At the expiration of this time they were of a long vermiform shape, obscurely segmented, and possessed 11 or 12 setigerous somites. Figures 60 and 61 represent the larvæ of eight days. The cilia have quite disappeared, and there are five setigerous somites. Each of these has a single uncinus in the lower ramus and in the upper rami 4, 4, 3, 2 and 1 respectively. The proboscis is already developed as a thickened region at the beginning of the alimentary canal and is actively protruded and withdrawn.

Figure 4, Plate XXIII, represents the young worm of 15 days, which possesses six setigerous somites. The larval eye-specks still remain, and the head is distinct. The dorsal pseudhæmal vessel is well developed. There is an especially glandular region of the stomach, extending from the third to the sixth setigerous somites. Beyond this point I have not followed the development, since the worms all died, probably from the lack of food.

Max Schultze has described (*l. c.*) the larvæ of the European *A. marina* (*piscatorum*) which are, in general, very similar to those of our species. But there is a narrow ciliated ring posterior and another anterior to the broad belt. The anterior extremity is much more acute, and the eyes lie in the broad belt of cilia instead of anterior to it. The somites become very definitely marked at an early stage, but the setæ do not appear until a far later period than in our species. Horst has also briefly described[1] the larvæ of a European species which agree closely with ours. Unfortunately I have not his paper at hand, for more exact reference.

The larvæ of *Clymenella* and *Arenicola* are essentially alike, so far as regards the distribution of the cilia; and they are of the larval type originally called *Telotrocha* by Johannes Müller, in which the cilia are arranged in two belts, one being præoral and the other near the posterior extremity. The belts in this case are, however, far less definite and concentrated than in the strictly free-swimming Telotrochæ, like the larvæ of *Nerine*, *Capitella* or

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[1] Tijdschrift der Nederl. Dierk. Vereenigung. Deel I, bl. 61.

Nephthys.[1] The belts are broad and the cilia short and weak. This modification of the Telotrochous type is evidently due to the circumstance that the free-swimming life is very brief, the larval development taking place chiefly in the gelatinous egg-mass. A very similar larva is that of *Terebella nebulosa*,[2] which likewise is protected within a gelatinous egg-mass. Claparède and Metschnikoff have also described a similar case—the larva of *Terebella Meckelii*—and have pointed out its significance. We find, in accordance with this view, that in the *Arenicola* larvæ which lead a brief free-swimming life the belts, in the earlier stages at least, are narrower and the cilia more powerful than in *Clymenella*, the larvæ of which never swim freely through the water. (This difference is not well illustrated by the figures.)

Chætopterus pergamentaceus, Cuvier.

This fine Annelide, for the identification of which I am indebted to the kindness of Professor Verrill, of Yale College, is common at Beaufort, and in the summer of 1881 I succeeded in fertilizing the eggs artificially and thus procuring the ciliated larvæ. The well-known researches of Johannes Müller, Busch and Max Müller have made us familiar with the later larval stages, but the segmentation of the egg and the early larval forms have not hitherto been described.

Adult worms of full sexual maturity were found on the sand-flats during the months of June and July. The ovaries are in the form of convoluted masses of long narrow flattened bands of a bright yellow color, which occupy a large part of the perivisceral cavity in the posterior region of the body. The spermaries occupy a similar position in the male (the sexes being distinct), but are of a creamy white color. The spermatozoa are of the ordinary tailed form.

The unfertilized ovum is a spherical body about .09 mm. in diameter; the vitellus is granular and opaque, though less so than in the preceding forms, and is surrounded by a very delicate membrane, which only becomes distinctly visible after fertilization.

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[1] Claparède und Metschnikoff. Zeitschrift für wiss. Zoologie, Bd. XIX, 1869.
[2] Milne-Edwards. Recherches Zoologiques, etc., Ann. d. Sciences Naturelles Sér. III, T. III, 1845.

My observations upon the segmentation, especially it its later stages, are somewhat fragmentary, but indicate a mode of development very similar in the main to that of *Clymenella*. A few minutes after fertilization the membrane begins to separate from the vitellus (Fig. 63). After a period usually of about thirty minutes the egg elongates slightly, becomes rather more transparent towards one end, and soon produces in succession two small clear direction cells (Fig. 64) at this end; the egg then becomes again spherical. The first cleavage, about twenty-five minutes later, divides the egg, as usual, into two unequal parts, the plane of division passing through the direction cells (Fig. 66). At the same time a rounded opaque prominence appears on the surface of the larger spherule, on the side opposite to the direction cells (Figs. 65, 66, *b*). This singular body, which is much larger and more opaque than the direction cells, subsequently fuses completely with the vitellus, and the part played by it in the development could not be ascertained. Its appearance is, possibly, due to pathological changes, but it appears to be always present, and I believe its formation to be a normal occurrence. Quatrefages observed a somewhat similar thing in the segmentation of *Sabellaria* (*Hermella*),[1] but normal and abnormal eggs are hopelessly confused in his paper.

After the first cleavage the egg passes into a very marked resting-stage (Fig. 67) in which the spherules flatten together much more completely than in the corresponding stage of *Clymenella*; this continues fifteen or twenty minutes. The second division is precisely like that of *Clymenella* or *Arenicola* (Figs. 68, 69); the plane of cleavage again passes through the direction cells, which therefore lie above the point where the four spherules meet. The egg then passes into a second marked resting-stage (Fig. 70) during which the peculiar prominence on the lower side of the egg fuses permanently with one of the spherules.

The third period of activity results, as before, in the separation of four micromeres (using this term for the sake of analogy) at the upper pole, where the direction cells are situated (Figs. 71, 72). These "micromeres" scarcely merit the name, for they are still larger than in *Arenicola*, being, in fact, but slightly smaller than

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[1] Mémoire sur l'embryogénie des Annelides. Ann. d. Sciences Naturelles, Sér. III, T. X, 1848.

the macromeres. They soon place themselves between the macromeres, and a third resting-stage results (Fig. 73).

In the next stage all the spherules divide at nearly the same moment (Fig. 74) and the inequality in size between the spherules is even less marked than before. The spherules again flatten down, and a fourth resting-stage follows (Fig. 75). Further stages in the segmentation are represented in Figures 76, 77, 78; in the last figure the outline of the entoderm is faintly visible. The inequality in size between the spherules becomes very slight after the stage represented in Figure 76, and I have not seen the large posterior macromeres observed in the other eggs.

The embryo gradually elongates and at some time between the twelfth and eighteenth hours becomes everywhere covered with cilia. These do not, however, perforate the egg membrane, as in the cases already described. During the segmentation the membrane separates more and more from the embryo and finally disappears.

The cilia very soon assume the arrangement shown in Figure 79 (18 hours). The cilia are somewhat longer over an area behind the middle of the body, thus forming an ill-defined belt in this region. There is a very definite anterior apical tuft of long cilia; at the posterior extremity the cilia are longer than those of the general surface, but they do not form a definite tuft. Six hours later (Fig. 80) the body is still more elongated, the mouth has appeared on the ventral surface in front of the belt of longer cilia, and a pair of eye-specks are present still further forward; they are upon nearly opposite sides of the body, though somewhat towards the dorsal side. The larva swims rapidly about, rotating, at the same time, on its long axis. The ectoderm and entoderm appear definitely separated, except at the extreme posterior end.

The larva of forty hours is represented in Figures 81 and 82 from the left and ventral sides respectively. The mouth is very distinct and leads into a distinct œsophagus. The ciliated belt is much more definite, and just behind it, on each side, is a long stout flagellum (f) which is usually in a state of rapid vibration. The anus is not yet formed. During the next twenty-four hours the larva rapidly elongates, the præoral lobe becomes distinct, and the three regions of the alimentary canal are clearly defined. The anus appears on the dorsal side just in front of a terminal papilla (Fig. 5, Pl. XXIII) which bears a tuft of long cilia. The

body becomes obscurely divided into three regions, viz: the very large præoral lobe, a middle region in which the stomach lies, and a posterior region including the short intestine. The belt of longer cilia encircles the middle region, but is now less definite than in the last stage figured. A second belt, as yet ill-defined, has, however, appeared on the posterior region. The cilia of both belts graduate into those covering the general surface. I could not see the flagella in the specimens figured, though they are certainly present in older specimens. The larva of five or six days are shown in Figures 83 and 84. They are much like those of three days, but the anterior belt of longer cilia has almost or quite disappeared, while the posterior belt is well developed. The flagella still remain, and in some specimens (Fig. 84) there are two or three, instead of one, on each side. The alimentary canal occupies almost the entire perivisceral space, which is reduced to a narrow cleft. The three regions of the former consist of a very wide rather thick-walled œsophagus, a large stomach with thick and glandular walls, and a very short globose thin-walled intestine. The mouth is enormously large and of a triangular form. From its posterior angle a narrow groove, lined with rather long cilia, runs backwards in the median line.

Figures 6 and 7, Plate XXIII, represent the larvæ of twelve days, the oldest raised from the egg. In general appearance they are considerably like the last stage, but the anterior belt of longer cilia is entirely atrophied, and the cilia over this part of the body do not differ from those covering the entire surface. The posterior belt, however, is now perfectly definite and greatly developed. It consists of a series of very long and powerful cilia, like those of the characteristic belts of other Mesotrochal forms. Towards the middle line on the ventral side these cilia gradually disappear, so that the belt is open below; it is very probable that new cilia are formed at this point. The cilia of the belt, except in being shorter, are much like the flagella of the last stage, and I was at first inclined to believe that the belt is formed by an extension of the flagella around the body. This point was not definitely settled; I think, however, that the belt is not thus formed, but is a further specialisation of the tract of longer cilia surrounding the posterior region of the body in the preceding stages.

Soon after the last stage described the larvæ all died. I have only once succeeded in taking an older larva at the surface, and of

this have, unluckily, no sketches. This larva agreed closely with the *Mesotrocha sexoculata* of Johannes Müller, which was shown by Max Müller to be a young *Chætopterus* — probably *C. Norvegicus*, Sars. In its general features it was similar to the larvæ just described, but there were two very distinct belts of powerful cilia. Hence it would appear that our larva, in the course of its further development, elongates and acquires a second belt of cilia which probably arises behind the first.

The ventral longitudinal ciliated groove is a somewhat interesting feature of the *Chætopterus* larva. It may perhaps be compared with the ventral ciliated region of the larvæ of *Clymenella*, *Arenicola*, and a number of other Polychæta, of *Echiurus* and of Oligochætous larvæ generally. In the embryo of *Euaxes* Kowalevsky observed a ciliated groove very similar to that of *Chætopterus*. In the genus *Protodrilus*, recently described by Hatschek, a similar groove is persistent throughout life; while in at least some other Oligochæta, as we know from Hatschek's beautiful researches, such a ventral ciliated groove becomes infolded to form a part of the ventral nerve cord. It is, however, quite possible that the ventral groove of *Chætopterus* has no significance in this direction, but is a special larval adaptation which simply plays a part in bringing particles of food towards the mouth.

The appearance of a provisional belt of cilia, which afterwards disappears and is replaced by another, may perhaps have some ancestral meaning.

Spiochætopterus oculatus, Webster. (?)

During the summer of 1879 numerous specimens of a Chætopterid larva were taken by the dipping-net at Fort Wool in the southern Chesapeake. These larvæ developed in the aquarium until they could be recognized as *Spiochætopterus*, or a closely allied genus, and for the sake of comparison with other larvæ of this family a brief description of them may be given. The larvæ were true Mesotrochæ, closely similar in every respect to the free-swimming young of *Telepsavus* as described by Claparède and Metschnikoff in the paper already referred to; hence a very brief description will suffice. My only reason for referring the larva to Sars's genus is that *Telepsavus* is not known to exist in our waters, while *Spiochætopterus* is abundant at some places on the eastern

shore of Virginia, as well as farther northwards. The "*Telep-savus*" larvæ were not identified with certainty and might with equal reason have been referred to *Spiochætopterus*.

The larva (Fig. 8, Pl. XXIII) is more or less elongated, though of exceedingly changeable form. As in *Chætopterus* there is a distinct præoral lobe, a fleshy bilobed lower lip and a large triangular mouth. The eyes are two in number, placed on nearly opposite sides of the body. Behind the eyes is a pair of very contractile short tentacles. A little way behind the middle of the body is a series of very powerful cilia encircling the body; they are borne on a thickened ring of the body-wall. This ring divides the body into two regions, the anterior of which contains nine somites, as shown by the groups of setæ; the posterior is imperfectly segmented and is terminated by a small appendage which resembles the terminal papilla of *Chætopterus*, or is more usually two-jointed. Just below the ciliated ring, on the dorsal side, are the rudiments of two pairs of branchiæ. The alimentary canal is very distinctly divided into the usual three regions—œsophagus, stomach, and intestine, the latter terminating in a dorsally placed anus. On the ventral side of the body, about opposite the seventh setigerous somite, is a glandular infolding of the body-wall.

In the oldest larvæ observed (Fig. 85) the body is much more elongate, and the anterior region and buccal segment have assumed the appearance of the adult. Two pair of branchiæ have appeared on each of the two segments behind the thickened ciliated ring; from the latter the cilia have nearly disappeared. The posterior region is much elongated and is distinctly segmented. The young worm has, in fact, attained practically the adult structure, though the middle or branchiferous region contains as yet only two somites. The œsophagus has elongated greatly, extending backwards nearly to the middle region. Figure 87 represents the peculiar stout seta of the fourth segment, and Figure 88 the ordinary form of setæ from the anterior region. The branchiæ are bilobed, and each lobe is furnished with a short series of powerful cilia.

The larvæ of all of the *Chætopteridæ*, so far as known, are true Mesotrochæ, and this type of larva is not known to occur in other groups of Annelides. These larvæ agree in so many other respects besides the arrangement of cilia, that it is not easy to avoid the conclusion that they must represent, to a certain extent, the ancestral type from which the various forms in this

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very peculiar family have been derived. However this may be, it is interesting to observe that the larvæ of *Spiochætopterus* (or of *Telepsavus*) with their single ciliated ring remain, throughout their larval existence, in a condition which is only temporary in the *Chætopterus* larva. The larva of *Phyllochætopterus*, like that of *Chætopterus*, has two ciliated rings (Claparède and Metschnikoff), though the adult is far more like *Spiochætopterus*. The case is of some interest as showing how readily the ciliation of larvæ may undergo modification, even within the limits of a small and well-circumscribed group, and quite independently of such conditions as parasitism or the special protection of the young in egg-masses like those of *Clymenella*. It would be interesting to observe whether the larva of *Phyllochætopterus* likewise passes through a temporary monotrochal stage.

Diopatra cuprea, Claparède.

A few observations upon the young of this species, made at Beaufort, may perhaps be worth describing for the sake of comparison with other Eunicid larvæ. The larvæ are found embedded in long strings of slimy jelly which may often be found attached to the tubes of the worm. In spite of numerous efforts, I have never procured the eggs in the early stages of development. The youngest larvæ observed (Fig. 89) were pear-shaped and without indication of segmentation. There are two widely separated eye-specks at the anterior extremity, and the body is peculiarly blotched with irregular spots of dark pigment. A very broad band of short cilia surrounds the middle region of the body, and just in front of this is another very narrow and ill-defined band. At the anterior end is a small apical tuft of cilia ; the narrow posterior extremity is surrounded by a narrow but pretty distinct ring. Thus the larva appears to be a slightly modified Atrocha, like other Eunicid larvæ.

In the next stage observed (Fig. 90) the body has elongated slightly, and the posterior region has become divided into five pretty distinct somites, each of which, except the last, has a tuft of cilia on each side. In other respects the ciliation is the same as in the last stage. We observe that the broad ciliated belt lies quite in front of the segmented part of the body and, therefore, presumably in the head region. At the posterior extremity of

the body are two slight protuberances which are rudiments of anal cirrhi. Figure 9, Plate XXIII, represents a considerably older larva from the same jelly-mass with the last. The segmented region has greatly elongated, the somites are very distinct and of about equal size, and the parapodia, with distinct dorsal cirrhi, have appeared. The eyes have moved backwards nearly to the middle of the head region. Nearly midway between them is the rudimentary median antenna, and just in front of each is a lateral antenna; the latter are much longer than the former. The cilia are arranged as in the last stage, except that the apical tuft and the anterior narrow ring have disappeared.

By the end of another week the young worms (Figs. 91, 92) have lost their cilia, left the jelly-mass and crawl about on the walls of the aquarium, especially on the side turned to the light. The body is vermiform, three new somites have appeared behind the five of the last stage, the parapodia are very prominent, the anal cirrhi elongated, and the head and buccal segment well defined. The cilia have disappeared, excepting a tuft in front of each parapodium behind the first. The median antenna has elongated considerably, the lateral ones still more so, and the latter have acquired the short basal portion present in the adult. Below the lateral antennæ a second pair have appeared which are still short and simple. On the upper side of the buccal segment are rudiments of the "tentacles" (or "tentacular cirrhi"). A parapodium in the anterior part of the body consists, at this stage, of a somewhat bilobed protuberance with a cirrhus above and below. The dorsal cirrhi are present in all the somites, diminishing in size to the last; the ventral cirrhi, however, become reduced behind the second somite to low rounded prominences. Rudiments of the branchiæ have appeared above the dorsal cirrhi of the sixth and seventh parapodia. The branchia is at first a simple ciliated cirrhiform appendage, which only in much later stages assumes the spirally branched structure of the adult. The subsequent development is very simple. The small frontal antennæ appear, two additional anal cirrhi grow out, the body elongates, and the young worm of a month old, except for the simple branchiæ, resembles the adult; it has at this age twenty-three setigerous somites. About the end of the second week the young worm leaves the jelly-mass and secretes a membraneous tube in which it thereafter dwells.

The *Diopatra* larva agrees pretty closely with that of *"Lumbriconereis* sp." described by Claparède and Metschnikoff as one of Müller's "*Atrochæ,*" also with the larva of *Eunice sanguinea* (Koch), and of the *Eunicid* larva described by Krohn and Schneider,[1] which appear to be likewise *Atrochæ.* I did not determine the precise relation of the mouth to the broad ciliated band, but the latter is always confined to the head region and the mouth appears at the posterior margin of the cephalic segment. Hence it seems very probable that the band either passes in front of the mouth or surrounds it, in which case the "Atrochal" larvæ of at least some Eunicidæ may be extreme modifications of the Telotrochous type, due, as in the case of *Clymenella, Arenicola* or *Terebella,* to the absence of a free-swimming mode of life. It appears very probable that *Diopatra cuprea,* like some other species of the Eunicidæ, is viviparous, or, at least, that the segmentation of the egg takes place within the perivisceral cavity of the parent. It is hardly possible otherwise that I could have failed to discover the segmenting eggs among the large number of egg-masses examined. This, if true, would perhaps explain some peculiarities of the development, such as the modification of the ciliation and the early and rapid development of the somites. In *Eunice sanguinea,*[2] which is certainly viviparous, the development is much abbreviated, and the somites and setæ appear very early. It is very probable that all of the "Atrochæ" are either young larvæ which have not yet acquired their characteristic larval features (*e. g., Chætopterus*), or forms which, like *Diopatra,* have become modified in accordance with special conditions, such as the absence of free-swimming life.

The young *Diopatra,* at the stage represented in Figure 89, is much like an unknown Eunicid larva observed by Krohn and Schneider, agreeing in the number and arrangement of the antennæ, the number of setigerous somites and anal cirrhi. It resembles also the young *Autolytus,* in the number, form and arrangement of the antennæ.[3] In the latter genus, according to Agassiz, the median antenna, though smallest, is first to appear. A further likeness lies in the fact that the "tentacular cirrhi" (append-

[1] Arch. für Anat. und Physiol., 1867.

[2] Koch. Neue Denkschrift der Allg. Schweiz. Gesellsch. ges. Naturwiss., Bd. VIII, 1847.

[3] Alex. Agassiz. Bost. Jour. Nat. Hist., Vol. VII, 1859–1863.

ages of the buccal segment) appear at a late period after both the antennæ and the dorsal cirrhi of the parapodia are well advanced in development. The same order in the development of the antennæ is followed by *Marphysa sanguinea*, according to Webster, the median one appearing first, then the upper lateral ones, and then the lower lateral. In view of the morphological importance which has been attached to the head appendages of the Annelides, it would be interesting to determine how far the order of their appearance is constant in different groups.

Our knowledge of the segmentation of the egg in the Polychæta is derived from the accounts of a considerable number of observers, among whom may be especially mentioned Milne-Edwards, Quatrefages, Sars, Claparède and Metschnikoff, Hæckel, Von Willemoes-Suhm, Giard, Stossich. The observations of Stossich have been already referred to. Those of Milne-Edwards give little more than the result of segmentation, without considering its details. Quatrefages was entirely led astray by his failure to distinguish between normal eggs and those which underwent pathological changes, as often happens when eggs are artificially fertilized. (I have unsuccessfully attempted this experiment with eggs of the same genus [*Sabellaria*], studied by Quatrefages, and can testify to the accuracy of his figures of the abnormal eggs.) He was thus led to certain results which he very justly asserts to be "tout nouveau dans l'histoire de l'embryogénie."

The general statement in regard to the segmentation made by Claparède and Metschnikoff in their admirable paper on Chætopod development is as follows: " Bei allen Chætopoden führt der Vorgang der Dotterklüftung zu der Bildung von zweierlei Dotterelementen, die sich von einander nicht nur in Bezug der Grösse, sondern auch durch das Ansehen, das Brechungsvermögen u. s. w. sehr bedeutend unterscheiden. Die Bildung dieses Gegensatzes der beiden Embryonalmassen rührt von der allerersten Zweitheilung des Dotters her, indem die erste Klüftungsfurche meist so angelegt wird, dass der Dotter in zwei ungleiche Hälften zerfällt. Beide klüften sich zwar weiter fort, die kleinere jedoch viel schneller als die grössere, so dass jene zur Bildung von sehr kleinen Furchungskugeln oder Zellen führt, welche die grösseren aus der Klüftung der anderen grösseren Hälfte herrührende

Kugeln allmählig umwachsen und einschliessen." [1] This account
is generally accepted, being given, for instance, almost *verbatim* in
Huxley's "Anatomy of Invertebrated Animals" and in the last
edition of Claus's "Grundzüge der Zoologie."   Hæckel's figures
of the segmenting eggs of *Fabricia* agree with this account and
are wonderfully like Claparède and Metschnikoff's figures of the
eggs of *Spio*, a widely different Annelide.   Without denying the
accuracy of these observations, it is nevertheless certain that in
the three genera described in this paper, one of which is very
different from the others, the ectodermic and entodermic parts of
the egg are not separated until long after the first cleavage.   No
separation is effected until the third cleavage, at least, when the
spherules can be distinguished as macromeres and micromeres;
and the complete dissociation of the layers is not, as I believe,
effected until near the close of segmentation.

   This error, if it be such, in regard to the early stages may
appear trivial, but, as already pointed out, it is one which ob-
scures the very close similarity between the Polychætous egg and
many others, and as such perhaps needs further observations for
its rectification.   Claparède and Metschnikoff drew attention to
the "sehr willkommene Uebereinstimmung" between the segmen-
tation of the Polychæta and of Hirudinea, but the correspondence
is much closer and more detailed than they supposed.   Thus the
earlier stages in the segmentation of *Clepsine* are closely similar,
even in details, except for the greater inequality of the micromeres
and macromeres, to those of *Clymenella*.   The egg of the Oligo-
chætous genus *Euaxes*, so carefully studied by Kowalevsky, is
still more like the Polychætous egg, since in this case the macro-
meres undergo division at a much earlier period than in *Clepsine*.
It may be worth while to compare the *Euaxes* egg with that of
Clymenella somewhat in detail.   After the second cleavage, the
egg is exactly like that of *Clymenella*, there being a large posterior
spherule, two smaller spherules, and one of intermediate size.
This stage is, however, differently attained, if Kowalevsky's very
explicit account is correct, inasmuch as the large posterior spherule
is the undivided smaller spherule of the first stage, while the three
others are produced by two successive divisions of the primary
larger spherule.   As in *Clymenella* four micromeres are produced at

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[1] Zeitschr. für wiss. Zool., Bd. XIX, p. 165, 1869.

the upper pole of the egg, though not at the same time. The posterior micromere is much larger and more opaque than the others, and gives rise to two mesoblasts as well as to ectoderm cells. Although it is not mentioned in the text, Kowalevsky's figures show that the separation of micromeres from the macromeres continues for a considerable period after the formation of the first four. The micromeres, with the exception of the posterior one already referred to, are at all stages much smaller and clearer than the macromeres, so that the limit of the ectoderm is always plainly visible. At no time, however, does any segmentation cavity appear, the invagination being typically epibolic.

As compared with the development of *Euaxes*, the peculiarities of the Polychætous segmentation depend upon the primary slight difference in size and constitution between the macromeres and micromeres and the speedy division of the former, so as to reduce this inequality still further. In *Clymenella* the inequality between the first four micromeres and four macromeres is much less than in *Euaxes*, in *Arenicola* still less, and in *Chætopterus* scarcely exists. In *Terebellides Strömii*, according to Willemoes-Suhm,[1] the segmentation is equal, but I gather from his somewhat fragmentary account that no segmentation cavity and no invagination were observed. Putting these facts together it would seem that the eggs of various Chætopods, if carefully studied, might show us within the limits of one group the actual steps in the conversion of invagination into delamination (*cf.*, p. 276). According to this view, the egg of *Serpula* represents the primitive form at the beginning of the series, having an equal segmentation, or nearly so, a large segmentation cavity, and undergoing an embolic invagination. As the entodermic portion of the egg became more and more loaded with food-yolk, the segmentation became more and more unequal, the segmentation cavity decreased in size and at length disappeared. This condition is retained by the egg of *Euaxes*. After this point, however, the segmentation becomes less unequal, owing, perhaps, to changes in the distribution of the food-yolk ; and the three genera described in this paper represent three stages in the return towards an equal segmentation. This return is not, however, accompanied by the reappearance of a segmentation cavity, so that an invagination is not possible, and

[1] Zeitsch. für wiss. Zool., XXI, 1871.

upon this fact has perhaps depended the acquisition of a mode of development resembling delamination. While the development of some Polychætous eggs, if my account is correct, has many of the features of an epibolic invagination, it is, on the other hand, nearly akin to a delamination like that of *Tetrastemma*[1] or *Clavularia*,[2] the main difference being that in the Annelide egg there is only a partial delamination and that is effected by succesive steps. An almost precisely similar case is that of *Tubularia* as described by Ciamician (*Zeitschrift für wissenschaftliche Zoologie*, XXXII, 1879), the eggs of which are more favorable for observation than those of Annelides, and which undergo a form of development almost exactly midway between epibolic invagination and delamination. His observations have not, however, been confirmed by other competent observers.

The only observer who has given a description of the later stages in accordance with that given above is Willemoes-Suhm, who says that in the eggs of *Terebellides zostericola* he could not satisfy himself that the micromeres envelop the macromeres, and adds : " In den ersten Furchungsstadien sah ich allerdings oft ungleiche Furchungskugeln und mit grösster Deutlichkeit .... aber niemals jene Stadien in denen das Vorhandensein der beiden Dotterelemente scharf und klar hervorgetreten wäre." On the other hand, in *Spirorbis*, "die kleineren Furchungskugeln umwachsen die grösseren."

Leaving this point, which must remain doubtful until a thorough study by the aid of sections can be made, certain other points are noteworthy. The greater size of the posterior spherule in the first stages is a curious fact which calls to mind the segmentation of many Molluscan eggs. The greater size of this spherule may be in part due to the storing up of mesoderm elements in it. That this is not, however, the only cause is proved by the case of *Euaxes*, where the preponderance in size of this spherule is quite as marked after the separation of the mesoblasts from it as before ; and where, moreover, a large part of the mesoderm does not come from this spherule at all. The principal cause seems to be a tendency towards the accumulation of food-yolk in this spherule, which is thereby retarded in its multiplication.

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[1] Hoffmann. Niederländisches Archiv, III, 1876-7.
Kowalevsky. Zool. Anzeiger, No. 38, 1879.

This tendency, if pushed still further, might lead to the formation of a true food-yolk, as Rabl and Brooks have shown it to have been formed in the Molluscan egg. It is, perhaps, worth noting that the Annelide egg corresponds in this respect very nearly to that stage in the evolution of a food-yolk which has not yet, according to Brooks, been discovered among the Mollusca.

The persistence in some cases of the chorion as the larval cuticle is a remarkable occurrence, entirely confined, so far as known, to the Chætopoda and Gephyrea, and by no means universal among them. Some doubt has been cast upon the accuracy of observations relating to this point; but it has been seen in so many cases and by so many different observers, that it is impossible not to accept it as a fact.

With regard to the nature of the various larval forms existing among the Polychæta, it is now generally admitted that, with perhaps one or two exceptions, they have little morphological importance; and that it is impossible to form any classification of them, based on the distribution of the cilia, which corresponds with the grouping of the adult forms. With the possible exception of the Mesotrochæ, which form a very distinct and well-defined group, all of the larval forms appear to be readily derivable from the Telotrocha; and in many cases the modifying conditions which have produced the change are obvious. The most important of these is the absence of a free-swimming pelagic life, and this, in turn, depends upon the provision made by the parent for the care of the embryo or larva during its early life. In the Oligochæta this provision is so perfect, both as regards food and protection, that a larval stage is entirely dispensed with, the cilia being reduced to a mere remnant. This condition is, however, but a step beyond such a larval form as that of *Diopatra* or *Eunice*, and it seems evident that the embryological differences between Polychæta and Oligochæta are due to purely adaptive conditions.

NOTE.—While this paper was in press, I received an important paper by Goette, entitled "Abhandlungen zur Entwickelungsgeschichte der Tiere (*sic*). Erstes Heft, Untersuchungen zur Entwickelungsgeschichte der Würmer. III, Ueber die Entwickelung der Chätopoden," [Leipzig, 1882, Leopold Voss.] The observations described in the paper were made by the author at Naples in 1880, and relate to the development of *Nereis* (*Hetero-*

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nereis) *Dumerilii* and *Spirorbis nautiloides.* The development of the latter species is somewhat like that of *Serpula,* an embolic invagination taking place. In the case of *Nereis,* the course of development is, in the main, similar to that which I have described in *Clymenella,* though differing in some important particulars which do not tend to confirm the conclusions which seemed to be indicated by my observations. The proportions of the four primary blastomeres and their division into four micromeres and four macromeres are almost precisely the same in both, though the relative sizes of the micromeres with respect to each other are slightly different. The micromeres multiply to form the ectoderm, *which does not receive additions from the macromeres.* The latter remain, indeed, quite undivided, except for the separation of a single large mesoblast from the largest of them, until a very late period when they have been entirely enclosed by the ectoderm and the blastopore has closed. They give origin then, in some unknown way, to much smaller true entoderm cells, and are themselves gradually used up as food material. Dr. Goette's observations lend no support to the view adopted at page 276 of this paper in regard to the separation of the germinal layers, an essential feature of which is that the ectoderm and entoderm are only gradually differentiated; for in the egg of *Nereis,* which is a far more favorable object for observation than those studied by me, the entire ectoderm appears to be included in the four primary micromeres which are separated at a single cleavage. In view, however, of the marked divergence between the development of *Nereis* and *Clymenella* in other respects, it is quite possible that they may differ materially in the formation of the ectoderm; and it is certain that the derivation of the entire ectoderm from the four primary micromeres in such a gastrula as that of *Nereis* is a somewhat exceptional occurrence.

I take this opportunity to state also that in a paper just published by Metschnikoff [Zeitschrift für wiss. Zoologie, December, 1881], Ciamician's observations on the development of *Tubularia* (see p. 294), are positively contradicted.

January, 1882.

EXPLANATION OF FIGURES.

PLATE XX.

FIGURE 1.—Unsegmented egg of *Clymenella torquata*, × 90.

FIGURES 2 to 4.—Formation of four primary blastomeres.

FIGURE 5.—Second resting stage from lower pole of egg.

FIGURE 6.—Same, from upper pole.

FIGURES 7 to 9.—Separation of the micromeres.

FIGURE 10.—Side view of an egg at the same stage.

FIGURE 11.—Third resting-stage, from upper pole.

FIGURES 12 to 17.—Fourth period of activity, from upper pole.

FIGURE 18.—The same, from the lower pole.

FIGURES 19 and 20.—From the upper pole, thirty minutes later, to show the separation of a small cell from the large posterior spherule.

FIGURE 21.—Oblique side view, ninety minutes later.

FIGURES 22 and 23.—Lower and upper sides of another egg at about the same stage.

FIGURE 24.—Lower side of another egg at about the same stage to show division of posterior spherule.

FIGURES 25 and 26.—Lower and upper sides of a still later stage.

FIGURE 27.—Side view of last.

FIGURE 28.—The same, still later.

FIGURE 28 *a*.—Longitudinal section of last stage, to show the large posterior spherules and the absence of a segmentation cavity.

FIGURE 29.—Embryo of fourteen hours; the large polygonal entoderm cells are visible in the anterior part.

FIGURE 30.—Later stage with the layers well differentiated.

FIGURE 31.—Larva about twenty-eight hours old, with anterior belt only.

FIGURES 32 and 33.—A few hours later, viewed from side and from dorsal surface respectively.

FIGURE 34.—Head of young worm possessing eleven setigerous somites.

PLATE XXI.

FIGURES 35 to 46.—Segmentation· of an egg of *Arenicola cristata* viewed from lower pole; time, 1 hour, 43 minutes; × 115.

FIGURES 47 to 53.—The same, seen from the upper pole; time, 1 hour, 30 minutes.

FIGURE 54.—Later stage, from lower pole.

FIGURES 55 and 56.—An older embryo from upper and lower sides respectively.

FIGURES 57 and 58.—Larvæ of 18 to 30 hours.

FIGURE 59.—Larva of three days.

FIGURES 60 and 61.—Larva of eight days, dorsal and lateral; in the latter the proboscis is fully protruded.

FIGURE 62.—The same; proboscis withdrawn.

FIGURE 89.—Young larva of *Diopatra cuprea* from jelly-mass; × 60.

FIGURE 90.—Older larva from same jelly-mass with last, the body is obscurely segmented; × 40.

FIGURES 91 and 92.—The same; young worms a week later, dorsal and ventral views; × 30.

PLATE XXII.

FIGURE 63.—Fertilized egg of *Chætopterus pergamentaceus;* × 180.

FIGURE 64.—The same, after extension of the direction cells.

FIGURE 65.—The same, after the first cleavage, from the lower pole.

FIGURE 66.—The same, from the side.

FIGURES 67 to 70.—Further development, from lower pole.

FIGURE 71.—Separation of the micromeres, from upper pole.

FIGURE 71 (second).—The same, viewed from side.

FIGURE 72.—Third resting stage, from upper pole.

FIGURE 73.—Fourth cleavage, from side.

FIGURE 74.—The following quiescent stage.

FIGURE 75.—Fifth stage of activity, from side.

FIGURES 76 and 77.—Later stages of the same egg.

FIGURE 79.—Larva of about 18 hours, more highly magnified.

FIGURE 80.—Larva of 24 hours, ventral view to show mouth.

FIGURE 81.—Larva of 40 hours, ventral.

FIGURE 82.—The same, from left side.

FIGURES 83 and 84.—Larvæ of 5½ days, lateral and dorsal views.

FIGURE 85.—*Spiochætopterus oculatus* (?), lateral view of advanced larva; × 40.

FIGURE 86.—The same; dorsal view of buccal segment.

FIGURE 87.—Peculiar seta from fourth segment; × 360.

FIGURE 88.—Seta of ordinary form, anterior region.

PLATE XXIII.

FIGURE 1.—Larva of *Clymenella torquata*, 60 hours old; × 125.

FIGURES 2 and 3.—The same, five days old; dorsal and lateral views; × 70.

FIGURE 4.—Larva of *Arenicola cristata*, four days old; × 215.

FIGURE 5.—The same; fifteen days old.

FIGURE 6.—Larva of *Chætopterus pergamentaceus*, 64 hours, from right side, × 295.

FIGURES 7 and 8.—Larva of twelve days, lateral and ventral views.

FIGURE 9.—*Spiochætopterus oculatus* (?), free-swimming larva, from left side; × 50.

FIGURE 10.—*Diopatra cuprea*, somewhat advanced larva from jelly-mass; × 60.

[NOTE.—The cilia in Figures 2 and 3 are represented much too long; in Figure 1 also they are longer than in nature].

THE ORIGIN OF THE EGGS OF SALPA. By W. K. BROOKS. With Plate XXIV.

In the summer of 1875 I enjoyed, through the kindness of Mr. Alex. Agassiz, the privilege of spending several months at his marine laboratory at Newport, R. I., and as specimens of Salpa were very abundant, I devoted myself, at Mr. Agassiz's suggestion, and with his assistance, to the study of their development. As the result of my investigations I was led to the conclusion that the eggs which undergo development inside the bodies of the chain Salpæ originate in an ovary contained in the body of the solitary Salpa, and that the latter is therefore the female and the chain Salpa a male; and that the life-history of Salpa is not a case of alternation of generations. As this was my first effort in the field of marine zoölogy, I should perhaps have hesitated to publish a view so directly opposed to the conclusions of the many famous naturalists who have contributed to our knowledge of Salpa; but as I was able to submit most of my specimens to Mr. Agassiz's examination, I relied upon his judgment, and published my results in a paper on "The Development of Salpa" in the Bulletin of the Museum of Comparative Zoölogy, No. 14.

In this paper I showed that the eggs of the chain Salpæ can be traced back to a club-shaped ovary which lies inside the stolon of the solitary Salpa, and which is present before the constrictions appear on the walls of the stolon, to mark off the bodies of the chain Salpa; and on pages 335–337 I gave the following summary of the subject: "Since the chain Salpa, at birth, always contains a single unimpregnated egg, organically connected with its body, and since this egg, as well as the resulting embryo, is nourished by the blood of the chain Salpa by means of a true placenta, and since no reproductive organs have hitherto been described in the solitary Salpa, it seems most natural to accept the view which has been generally held since the time of Chamisso's famous paper ; that is, that Salpa presents an instance of 'alternation of generations.' This view, in its most modern form, may be stated as follows: '*It is now a settled fact that the reproductive organs*

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*are found only in the aggregated individuals of Salpa, while the
solitary individuals, which are produced from the fertilized eggs, have,
in place of sexual organs, a bud-stolon, and reproduce in the asexual
manner exclusively, by the formation of buds.   Male and female
organs are, as far as we yet know, united in the Salpæ in one indi-
vidual.   The Salpæ are hermaphrodite.'* (*Leuckart.   Salpa u. Ver-
wandten*, pp. 46, 47).

"When, however, we trace backward the history of one of the
individuals which compose a chain, and find that the egg is present
at all stages of growth, and has exactly the same size and appear-
ance as at the time when it is impregnated: when we find one
organ after another disappearing until at last we have nothing but
a faint constriction in the wall of the tube, indicating what is to
become the animal, the conclusion seems irresistible, that the ani-
mal, which has as yet no existence, cannot be the parent of the egg
which is already fully formed.

"The life-history of Salpa may then be stated briefly as follows:

"The solitary Salpa — female — produces a chain of males by
budding, and discharges an egg into each of these before birth.

"The eggs are impregnated while the zooids of the chain are
very small and sexually immature, and they develop into females
which give rise to other males in the same way.

"Since both forms are the offspring of the female, the one by
budding and the other by true sexual reproduction, we have not
an instance of 'alternation of generations,' but a very remarkable
difference in the form and mode of origin of the sexes."

After I had finished my observations and while I was writing
my paper, Kowalevsky's paper on the development of Pyrosoma
(*Zeit. f. wiss. Zool.*, 1875) was published, and in this he also states
that the eggs of Salpa arise in an ovary which is contained in the
body of the solitary Salpa.   "Bei den Salpen giebt es bekanntlich
zwei Generationen, in der einen entwickelt sich der aus vielen
Eikeimen bestehende Eierstock, welcher in den Stolo hineingeht,
und sich hier zu je einem einzigen Eie vertheilt, sodann die einzelnen
Knospen- resp. Ketten-Salpen, in welchen weiter aus diesem Eie
ein Embryo entsteht, wieder mit einem aus mehreren Eikeimen
bestehenden Eierstock."   Kowalevsky fails, however, to perceive
that the origin of the eggs in the body of the solitary Salpa ren-
ders this a female, as he goes on to say: "Bei Pyrosoma enthält
jede Knospe auch wie die Kettensalpe das einzige grosse Ei zur

unmittelbaren geschlechtlichen Vermehrung, und wie die *Salpen-Amme* den Eierstock mit vielen Eikeimen zur Bildung der *Geschlechtsorgane* der künftigen Knospen."

If Kowalevsky's statement and my own, that the eggs originate in the body of the solitary Salpa, are based upon sound observation, I do not see how my conclusion that the solitary Salpa is a female can be rejected, for that animal in which the eggs first appear, as eggs, is certainly their mother; but soon after my paper appeared Salensky published three very thorough and exhaustive papers on the development of Salpa, "Ueber die Entwickelungsgeschichte der Salpen," Zeit. f. wiss. Zool., XXVII, pp. 179–237, Taf. XIV–XVI; "Die Knospung der Salpen," Morph. Jahrbuch, III, 4, and "Ueber die Entwickelung der Hoden und über den Generationswechsel der Salpen," Zeit. f. wiss. Zool., XXX, 275–293, Taf. XIII, and in these he says that Kowalevsky and I are wrong in our statement that the eggs do originate, as eggs, in the solitary Salpa. He acknowledges that the egg-cells can be traced back to a mass of cells at the base of the stolon, but he claims that they do not become eggs until they pass into the bodies of the chain Salpa; that what I have called the ovary is not an ovary at all, but simply a mass of undifferentiated embryonic cells, which gives rise to the ovaries of the chain Salpæ and also to their digestive organs.

This discrepancy between his observations and my own has rendered me very desirous of an opportunity to go over the ground once more, to re-examine the subject for myself. For several years I have been unable to do so, but last summer I requested Professor Baird to try to obtain specimens of Salpa for me, and at his request Professor Verrill collected a supply of specimens of a very large new species, off Nantucket Island. These were carefully preserved for histological work, by Prof. Lee, and were sent to me in the fall. I found that they were in excellent condition for microscopic work, and I very soon obtained transverse sections through the base of a very young stolon, showing fully developed ovarian eggs in Salensky's "endoderm."

The great size and perfect preservation of the specimens enabled me to obtain sections which have the greatest possible clearness, and I soon found that while Salensky's figures give the general anatomy of the stolon as shown in transverse sections, longitudinal sections show that his account is very far from complete, and

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is, in some very important features, incorrect. I therefore attempted to trace anew the whole history of the chain Salpa. This involved the preparation of several thousand sections, and as it was of the greatest importance that every section should be perfect, Dr. I. Bermann, of Baltimore, very kindly consented to stain and imbed the specimens for me, by his process, and then, with the greatest patience and interest in the work, to cut and mount the necessary sections. I accordingly now have the material for a very thorough description of the stolon and of the formation of the chain Salpa, but as the preparation of a fully illustrated paper will require considerable time, I have prepared this account of those of my observations which bear upon the origin of the eggs, and upon the question of alternation.

Salensky has given a very complete and clear statement of the point at issue, and I will quote it, in full, as an introduction to the description of the figures.

"Nachdem wir eben die Entwickelung der Salpenhoden kennen gelernt haben, können wir nun auf Grund der hier auseinandergesetzten Thatsachen, sowie deren, welche von mir an einem anderen Orte über die Entwickelung des Eierstocks mitgetheilt worden, zur Discussion der Frage übergehen: gehört die Entwickelung der Salpen zum Typus des Generationswechsels, oder muss dieselbe an irgend eine andere Fortpflanzungsart angereiht werden? Bevor wir aber zur Kritik der darüber bestehenden Meinungen schreiten, müssen wir darauf Acht geben, dass bei der Knospung der Salpen einige Eigenthümlichkeiten vorkommen, welche der Salpenfortpflanzung einen ganz besonderen Character geben. Das Wesentlichste von diesen Eigenthümlichkeiten besteht in der sehr frühzeitigen Entwicklung der Eier in der Salpen-Knospe; es ist bekannt, dass jede Kettensalpe noch lange bevor die Kette vom Mutterindividuum sich lostrennt, ein Ei bekommt, welches bereits in einem ziemlich reifen Zustande vorhanden ist. In keinem der bekannten Fälle des Generationswechsels treffen wir eine so frühzeitige Entwicklung der Geschlechtsproducte, und dieser Umstand hat, wie es scheint, als Beweggrund für die Annahme gedient, dass die solitären Salpen, welche man bisher als ungeschlechtliche Formen betrachtet hat, weibliche Individuen sind, dass sie aber ihre Eier in die von ihnen selbst producirten Kettensalpen ablegen. Ist diese Annahme richtig, so muss die Fortpflanzung der Salpen nicht als ein Fall des Generations-

wechsels, sondern als eine ganz besondere Fortpflanzungserschei-
nung betrachtet werden. Solche Meinung wurde von Brooks in
seinen von mir schon mehrmals citirten Aufsätzen über die Ent-
wicklung der Salpen ausgesprochen.

"Nach der Meinung von Brooks hat die Fortpflanzung der
Salpen eine Analogie mit der der Bienen; er findet diese Analogie
in der Art der Entwicklung der Geschlechter bei diesen beiden
Thiergruppen.

"Wenn man selbst mit Brooks darin übereinstimmt, dass die
solitären Salpen weibliche, die Kettensalpen männliche Individuen
darstellen, so kann man diese Analogie nur insofern bestehen
lassen, dass die Kettensalpen, wie die männlichen Bienen, ohne
Befruchtung durch ungeschlechtliche Vermehrung entstehen, wäh-
rend die solitären Salpen, wie die weiblichen Bienen, aus dem
befruchteten Ei sich entwickeln. Weiter geht die Analogie nicht,
und der wesentlichste Punct der Salpenvermehrung, namentlich
das hypothetische Ablegen der Eier von solitären Salpen in die
männlichen Kettensalpen, bleibt ohnedem ganz isolirt, denn im
ganzen Thierreich treffen wir keine dem analoge Fortpflanzungs-
erscheinungen. Wo finden wir in der That eine Vermehrung, bei
welcher eine geschlechtliche Form ihre Eier in die Knospen,
welche sie selbst producirt, ablege? Um eine derartige Fortpflan-
zungsweise für die Salpen zuzulassen, müsste man zuerst beweisen,
dass die solitären Salpen wirklich die Eierstöcke oder deren Homo-
logon besitzen, und dass die Eier der Kettensalpen aus diesen
Eierstöcken entstehen. Dies wurde durch keine Untersuchung
bewiesen. Brooks bestrebt sich zu beweisen, dass bei den Asci-
dien einige den bei Salpen vorkommenden analoge Fortpflan-
zungserscheinungen sich finden, und dass die Eier dieser Thiere
genau in derselben Weise, wie er es für die Salpen angiebt, von
einer Generation in die andere übergehen. Er sagt darüber Fol-
gendes: 'Die Zooiden der meisten Tunicaten sind hermaphroditisch
und entwickeln Eier aus ihrem eigenen Ovarium, aber, wenigstens
bei Pyrosoma, Perophora, Didemnium und Amauricium, ist das
Ei, welches die Befruchtung und Entwickelung in dem Körper
des Zooids erfährt, nicht aus dem eigenen Ovarium, sondern von
dem der vorhergehenden Generation, und die Eier, welche im
Körper der zweiten Generation erzeugt werden, müssen in die
Körper der Zooiden der dritten Generation übergehen, bevor sie
befruchtet werden können' (*Arch. f. Naturg.*, 1876, Heft 3, p. 353).

"Ehe ich auf eine Behandlung der von Brooks angeführten Ascidien weiter eingehe, will ich hier einige Bemerkungen über die Analogie der Entwicklung der Salpen und Ascidien im Allgemeinen vorausschicken. Die Analogie, welche hauptsächlich die Knospungserscheinungen dieser beiden Tunicatengruppen betrifft, wurde von mir in meiner früher citirten Schrift 'über die Knospung der Salpen' berücksichtigt. Sie besteht meiner Meinung nach darin, dass an der Bildung des Keimstocks oder der Stolonen der Salpen, so gut wie der Ascidien, die Derivate aller Keimblätter theilnehmen. Diese Analogie wird aber bei der Bildung der Athemhöhle dieser beiden Tunicatenordnungen wesentlich gestört. Bei den Ascidien bildet sich die Athemhöhle als eine unmittelbare Fortsetzung des gleichnamigen Gebildes des Mutterthieres, bei den Salpen ensteht dieselbe aus einer besonderen Anlage, welche zugleich als Anlage des Eierstocks dient. Bei den Salpen giebt es keine besondere Eierstocksanlage, und das ist ein sehr wesentlicher Umstand, welcher den Grundsätzen der Brooks'schen Theorie widerspricht. Wenn der Zellenklumpen, aus welchen die Eierstöcke und die Athemhöhlen der Kettensalpen entstehen, nur die Anlage des Eierstocks darstellte, so könnte man denselben unter gewissen Umständen als Eierstock der solitären Salpen betrachten, vorausgesetzt, dass er bei den solitären Salpen im unentwickelten Zustande existirt und erst in der Folge der Generation resp. bei den Kettensalpen zur vollen Entwicklung kommt; man könnte aus diesem Grunde die solitäre Salpe für ein weibliches Individuum halten. Ist aber einmal bewiesen, dass im Keimstock der Salpen keine besondere Eierstocksanlage existirt, so können wir den Zellenklumpen, welcher nur theilweise in den Eierstock der Kettensalpen übergeht, nicht als Eierstock betrachten. Bei den Ascidien ist aber, nach den Angaben von Kowalevsky u. A., eine besondere Eierstocksanlage vorhanden, welche von der Anlage der Athemhöhle vollkommen different ist. Das ist der wesentlichste Unterschied in der Fortpflanzungsgeschichte beider Tunicatengruppen, welcher genügt, um zu beweisen, dass das Eierstocksrohr der Ascidien mit dem Entoderm der Salpen nicht homolog ist.

"Aus allem oben Gesagten kann man den Schluss ziehen, dass die solitären Salpen keinen Eierstock besitzen; da bei ihnen gleichzeitig kein Hoden nachweisbar ist, so können dieselben als Formen der ungeschlechtlichen Generation betrachtet werden.

"Die Annahme der ungeschlechtlichen Natur der solitären Salpen kann schon allein für die Aufrechthaltung der früheren Theorie des Generationswechsels genügen, welche offenbar die anderen Theorien, wie z. B. die von Brooks und Todaro, ausschliesst, und allein die Fortpflanzungsverhältnisse der Salpen in richtiger Weise darstellt." (Salensky. *Entwicklung der Hoden und über den Generationswechsel der Salpen,* pp. 283–5).

Salensky's earlier paper (*Die Knospung der Salpen*) contains an excellent account of the general anatomy of the stolon, so far as it can be made out from transverse sections, but longitudinal sections would have shown him that the digestive tracts of the chain Salpæ appear very much earlier than he states, and that they are derived, not from his "endoderm," but after the analogy of other Tunicates from his "Athemrohr," with which, at first, they freely communicate.

If his specimens had been sufficiently well preserved to admit of the examination of very thin sections with high powers he would have found also that his "endoderm" was not simply an "Eierstocksanlage" of embryonic cells, but a true ovary with fully developed ova.

In support of these statements I shall now describe a few of my own sections.

Figure 1, is a transverse section of the base of a very small stolon, and represents the same stage as Salensky's Figure 3. Figure 2 is a similar section of a somewhat older stolon, and is at about the same stage as Salensky's Figure 10.

The stolon from which this section was cut had been a little twisted, either by its own curvature or by the action of the preserving fluid, so that the two sides are not symmetrical. Figure 3. is a transverse section of an older and larger stolon, upon which the constrictions marking off the bodies of the chain Salpæ had been formed, and it corresponds pretty closely to Salensky's Figure 12. Like Figure 2 it is a little unsymmetrical. Figure 4 is a vertical longitudinal section of the same part of another stolon at the same stage, giving, as it passes through the bodies of the chain Salpæ, what is equivalent to a series of vertical sections of Figure 3, along the numbered lines. That is the line 1–2, in Figure 4, shows what would be seen in a section of Figure 3, perpendicular to the plane of the paper on the line 1–2. The line 3–4, in Figure 4, shows, in the same way,

what we should have in a section of Figure 3, along the line 3–4, and so on.

Figure 5, is a highly magnified view of a fragment of a section through the ovary *h*, of Figure 1, and Figure 6 is a longitudinal section through the ovary of another stolon : the portion crossed by the line 1, being of nearly the same age as the ovary *h*, of Figure 1 ; the part crossed by the line 2, of about the same age as the ovary of Figure 2, and that crossed by the line 3, of about the same age as that of Figure 3.

In all the figures, *a*, is the outer wall or ectoderm of the stolon or of the chain Salpæ ; *b*, is the nerve tube, the Nervenrohr, *N*, of Salensky's figures and the tube *y'* of Figure 28 of my first paper. I there spoke of it as a second ovary, but my sections, as well as those of Salensky show that this was an error : *c* and *g* are the sinus tubes 1, 1, of Figure 28 of my first paper, Salensky's " Blut-räume," Br. They seem to have been overlooked by Kowalevsky ; *d*, is the " central tube," Figure 28, 2, of my first paper, Salensky's " Athemrohr," *Ar*, and apparently Kowalevsky's " Darmrohr " ; *e* and *f* are its thin upper and lower walls ; *h*, is the ovary, the " ovary *y* " of Figure 28 of my first paper ; Salensky's " en-doderm *En*," and Kowalevsky's " Eierstocksrohr " ; *i*, *i*, the " thickened edges 3, 3, of inner tube " of my original Figure 28, the " Mesoderm *Ms* " of Salensky, and the " Kloakalröhren " of Kowalevsky.

The greater part of the ovary *h* of Figure 1, is made up of a granular ground-work in which are numerous transparent ovvidal nucleated bodies, which at first sight appear to be cells. In Sa-lensky's Figures 3, 4 and 7, they are represented as a compact mass of cells, in contact with each other, and at first sight they do appear to cover the whole surface of the section, but more careful examination with a high power shows that only a few of them lie in the plane of the section and that these are widely separated by the granular substance, while between them, others at a lower level are seen through this substance. As it is very difficult to represent, at the same time, transparency and obscurity of outline, in black pen drawing for reproduction by photo-lithography, I have only drawn, in Figure 1, those which were in the plane of the section. Between them there are very faint straight lines, mapping out the granular substance into polygonal areas, with one of the transpa-rent bodies near the centre of each. When a very thin section is

examined with a high power, Figure 5, each of the oval trans-
parent bodies is seen to be a germinative vesicle, with a nucleolus
suspended in its cavity by a protoplasmic reticulum of fine
branching threads; and surrounded by a granular layer of yolk
which is rendered angular and polygonal by the pressure of adja-
cent eggs. I have obtained a complete series of sections showing
the eggs at every stage, from the one just described, up to the time
when the single eggs are attached, by their gubernacula, to the
wall of the branchial sac of the chain Salpa, and no one who ex-
amines the series, can doubt for an instant that the bodies in Figure
1, not only develop into eggs, but that they are actually eggs,
differing very slightly from the mature egg.

As we pass along the stolon, we find that the germinative vesicle
becomes a very little larger, the yolk grows more abundant and
the outline of each egg becomes more distinct and spherical, but
these slight changes are all, and before any traces of constrictions
appear on the surface of the stolon they have their mature form.
The ovary is surrounded by a layer of epithelial cells which are
thin and flattened at the sides, as shown at *m* in Figure 5, while
at the point where the ovary touches the ectoderm they form a
thicker layer, Figure 6, *m*, which however, is only one cell deep.
This layer gradually increases in thickness, as shown in Figures 2
and 3, and when the constrictions appear and mark off the bodies of
the chain Salpæ it becomes folded into a series of pouches, which
form the egg follicles, the so-called ovaries of the chain Salpæ.
These pouches are what Salensky has wrongly interpreted as the
developing digestive tracts of the chain Salpæ, but we shall see
farther on that the digestive tracts follow the analogy of the other
Tunicates and are developed from the walls of the large central
chamber of the stolon, Figure 1, *d*, Salensky's Athemrohr. Near
the internal surface of the ovary the epithelial layer changes its
character, as shown at *m*, in Figure 5 and 6, and on a smaller
scale at the top of *h*, in Figures 1 and 3. It becomes several cells
thick, and the cells become oval, transparent, with conspicuous
nuclei, and they resemble the germinative vesicles of the ova-
rian eggs in general appearance except that they are smaller.
Figure 3 and *n* of Figure 6, show that this layer gradually dis-
appears as we pass towards the free end of the stolon, and when
the constrictions appear it is very thin or absent. Figure 1, *n*,
shows that the eggs nearest the internal edge of the ovary are

smaller than those near its outer end, and this fact, together with
the fact that the layer n, is thickest at the base of the stolon, and
gradually disappears towards the free end, seem to show conclu-
sively that n is the germinal epithelium, the cells of which become
converted into eggs, which form a compact mass entirely filling
the lumen of the organ.

Full force cannot be given to the evidence without figuring the
eggs at all stages up to the time when the Salpa chain is discharged
from the body of the solitary Salpa, but I trust that the sections
which I have figured and described are enough to show con-
clusively that the body, h, of Figure 1, is not an "Eierstock-
sanlage" but a true ovary, and that the cells, o, Figure 5, are not
undifferentiated embryonic endoderm cells, but ova. As no one
has ever claimed that the so-called ovary of the chain Salpa gives
rise to eggs, or ever contains more than a single egg, and as the
single egg which it does contain, is present, not as an embryonic
cell, but as an egg, in the ovary of the solitary Salpa, before the
chain Salpa comes into existence, I do not see how it is possible to
refuse to accept the conclusion that the solitary Salpa is the true
female, even if it were true that the ovary does also give rise to
the digestive organs of the chain Salpæ, but this is not the case.

Figure 3 is a transverse section of a stolon on the sides of which
the constrictions are just beginning to appear. It is at almost
exactly the same stage as Salensky's Figure 12, although there are
slight differences, which are no doubt due to the fact that the two
sections are not from the same species. The most conspicuous
difference is due to the fact that the central tube, Figure 3, d, is
widely open, while in Salensky's Figure 12, its upper and lower
surfaces are almost in contact and the cavity, Ar, is nearly ob-
literated.

The sides of the stolon are formed by two thickened masses,
k, k, which, according to Salensky are masses of mesoderm cells,
Figure 12, Ms. In transverse sections they do have much the
appearance shown in his figure, but very careful examination of a
favorable section will show traces of a central cavity, shown on the
left in Figure 3, opening into the central cavity or "Athemrohr," d.
Longitudinal sections of the stolon show that, far from being an
unorganized mass of mesoderm cells, the body, k, actually has a
very complicated structure, and consists of a series of flat pouches,
the digestive tracts of the chain Salpæ, which open into the central

tube, *d*, and which are separated from each other by infoldings of the outer wall or ectoderm of the stolon.

These pouches are flattened so that it is almost impossible to study them in transverse sections, but through the skill of Dr. Bermann I have been able to get a complete series of sections through the stage of Figure 3, in a vertical plane, perpendicular to the paper. It is not necessary to figure all these sections for all the points are shown in a single longitudinal section. A longitudinal section passes, of course, through the bodies of a whole series of chain Salpæ, and as the stolon is always more or less curved, such a section will not follow its central axis, but will cut the bodies of the chain Salpæ at different distances from the centre, and it is plain that a section passing very obliquely through the stolon from one side to the other, would give, on each side of the central axis, what would be, in effect, a series of parallel and consecutive sections of the body of a single chain Salpa, although actually, no two of these sections would pass through the body of the same individual. Half of such a section is shown in Figure 4, and the vertical numbered lines indicate the axis of sections in the planes of the numbered lines of Figure 3.

Along the line 1–2 we have first the ectoderm *a* of Figure 3; then the upper blood-tube *c*; then the upper wall *e* of the central tube or "Athemrohr;" then the cavity *d* of this tube; then its lower wall *f*, and the lower blood-tube *g*, nearly filled by the ovary *h*, which is made up, as in the transverse section Figure 3, of an internal germinal epithelium, a mass of eggs, and a peripheral layer of epithelial cells. Along the line 3–4 we have the same structures in the same order, but we also have at the top of the figure a section of the nerve-tube *b*. As this is now broken up, by the constrictions, into a series of chambers, it appears as a tube, in longitudinal as well as in transverse sections. Near the middle of the central tube *d* we also have a small slice from the edge of the mass *k* of Figure 3, Salensky's mesoderm. Along the line 5–6 we have the ectoderm *a*, the nerve-tube *b* and the blood-tube *c* at the top of the figure, but below the latter we have, in place of the central tube *d*, a section through the base of the mass *k*, and this is now seen to consist of a central cavity *p*, which opens into the tube *d*, and is bounded on each side by a single layer of endoderm cells, which are continuous, around the edges of the opening, with the walls of the central tube. Along the

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line 7–8 we have this digestive pouch *p* as before, but between it and the pouch of the next chain Salpa we have a fold of ectoderm, *a*, and between this and one side of the digestive pouch, a section of a structure, *i*, which is, in all probability, a portion of the cloacal tube *i* of Figures 1 and 2. Along the line 9–10 and the line 11–12, we have the same structures, but the bodies of adjacent chain Salpæ are more perfectly separated from each other than they are nearer the axis of the stolon.

We have obtained hundreds of sections similar to the one shown in Figure 4, and the presence of the digestive pouches at the stage shown in Figure 3, and their communication with the central tube, are points upon which there can be no doubt.

Some of the sections show these points even more clearly than Figure 4, and the only reason for selecting this section is that the stolon from which it was cut was distorted almost exactly like Figure 3, so that 3 and 4 not only resemble each other in general structure, but in more minute features as well.

In the passage which has been quoted, Salensky says, that if the "Eierstocksanlage" did not also give rise to the digestive tracts of the chain Salpæ, and if it contained true eggs, instead of egg cells, the solitary Salpa might properly be regarded as a female, and as I have shown that the digestive organs are really formed from the central tube, while the ovary does contain true eggs, I think that the female nature of the solitary Salpa may be regarded as proven, and that we must conclude that we have in Salpa not a case of the alternation of an asexual with an hermaphrodite sexual generation, but simply a great and very anomalous difference in the form and origin of the sexes.

While writing this paper I have received two papers on the development of Salpa (*Neue Untersuchungen über die embryonale Entwickelung der Salpen, Vorläufige Mittheilung, von Prof. W. Salensky.* Zool. Anzeiger, No. 97 u. 98, Nov. 28th, 1881, and *Mémoire sur les membranes embryonnaires des Salpes, par le Dr. J. Barrois.* Journal de l'Anatomie et de la Physiologie, Dec. 28th, 1881), but as neither author treats of the origin of the chain Salpa or of the eggs, I have made no reference to them.

BALTIMORE, January 27th, 1882

EXPLANATION OF PLATE XXIV.

All the figures are from the stolon of an undescribed species of Salpa, from the Atlantic, off Nantucket Island.

The reference letters ·have the following significance in all the figures:

- *a.*   Outer tube of stolon or ectoderm of chain Salpæ.
- *b.*   Nerve-tube or ganglia of chain Salpæ.
- *c.*   Upper blood-tube.
- *d.*   Central tube.
- *e.*   Upper wall of central tube.
- *f.*   Lower wall of central tube.
- *g.*   Lower blood-tube.
- *h.*   Ovary.
- *i.*   Cloacal tube.
- *k.*   Lateral thickenings of central tube of stolon to form the digestive cavities of the chain Salpæ.
- *m.*   Epithelium of ovary.
- *n.*   Germinal epithelium of ovary.
- *o.*   Eggs.
- *p.*   Digestive cavities of chain Salpæ.

FIGURE 1.—Transverse section through the base of a very young stolon. Zeiss, D, 2.

FIGURE 2.—Transverse section of an older stolon, a little further from base. Zeiss, D, 2.

FIGURE 3.—Transverse section still further from base.

FIGURE 4.—Half of an oblique vertical section through Figure 3. Zeiss, D, 2.

FIGURE 5.—Fragment of a very thin section of the ovary of Figure 1. Zeiss, F, 2.

FIGURE 6.—Longitudinal section of the ovary of a young stolon.

The line 1 passes through a portion which is in nearly the same stage as *h* of Figure 1; the line 2 through a portion like Figure 2, *h*, and the line 3 through a portion like Figure 3, *h*.

OBSERVATIONS ON THE MEAN PRESSURE AND THE CHARACTERS OF THE PULSE-WAVE IN THE CORONARY ARTERIES OF THE HEART.

By H. NEWELL MARTIN, M.A., M.D., D.Sc., and W. T. SEDGWICK, Ph.D. With Plates XXV, XXVI and XXVII.

While for a considerable number of years careful studies of the blood-flow in various arteries of the mammalian body have been made under different conditions, the arteries of the heart itself have remained in an exceptional position. The average pressure and the pulse characters in them have been unknown, in spite of the recognized fact that great interest and importance belong to their study.

The following pages give an account of experiments undertaken with the object of gaining some knowledge of these points, and contain, we believe, a description of the first successful attempt to record graphically, as in other arteries, the blood-pressure and its variations in the arteries of the heart. They were begun in the first place for the purpose of testing the theory of Thebesius—a theory independently propounded and warmly supported in recent times by Brücke, and others, concerning the physiology of the aortic semilunar valves. According to this theory, during ventricular systole the thin flaps of the valve are pressed upwards and cover the mouths of the coronary arteries, completely closing them, so that blood can enter those vessels only during the time of ventricular diastole, and during that small portion of the systolic period which is occupied by the valve in travelling from its diastolic position across the mouth of the aorta, to its systolic position against the aortic wall and over the mouths of the coronaries. Observations on the spirting of blood from a cut coronary artery have shown this to be synchronous with systole of the ventricle; but to the value of these observations Brücke[1] has raised two objections. First, that merely opening the pericardium is enough to destroy the normal

[1] *Vorlesungen*, 1881, S. 185.

action of the heart and consequently of the valve under consideration; and, second, even supposing it does not thus interfere, that the brief period before the valve-flap closes over the coronary (and during which the coronary blood must share the rise of aortic pressure due to commencing systole) is quite sufficient to cause a systolic spirting, especially if the outflow from the heart vessels is hindered by the increasing cardiac contraction compressing the smaller branches in the heart walls. The first objection seems to us trivial when only a small slit over the artery is made in the pericardial sac; but the second is more formidable. We know that in the first stage of the systole, before the valve can close over their mouths, a rise of pressure must take place in the coronary trunks. To urge, therefore, that mere observation of spirting from the cut end of such a trunk can settle the question, is to claim that the unaided eye can determine whether that spirting is due to aortic pressure extending all through the cardiac cycle, or to the same pressure exerted only in the early portion of the systolic period. Considering how little time is needed for a complete contraction, this is clearly impossible. Again, with our previous ignorance of the events transpiring in the cardiac vessels, and with no experimental evidence of increase or diminution of resistance in the smaller twigs during the systole, it is, as Brücke urges, quite conceivable that some spirting might occur during that period, owing to the simultaneous effects of hindrance to the outflow and of increased pressure exerted upon the vessels by the contracting tissue. In a case like this, which calls for accurate observation and comparison, the graphical method is the only satisfactory one; and as numerous attempts to settle the question at stake, based on other methods, have given rise to great diversity of opinion, we set to work to obtain, if possible, simultaneous records of the blood-pressure and pulse-waves in the coronary and carotid arteries.

Although the opposite view has from time to time been upheld by many anatomists and physiologists, nevertheless Brücke has so skilfully defended his theory that it is accepted by many physiologists to-day; we, at least, prepared for our experiments with a decided leaning toward his view, and began work in the hope of establishing it more firmly. The results of our investi-

gation, however, have forced us to believe that the semilunar
valves do not act as Brücke supposes, and that his theory is no
longer tenable. Apart, however, from this point, we venture to
believe that the work possesses interest of its own ; and that the
discovery that it is quite possible to get tracings of the blood-
pressure in the arteries of the dog's heart, lays open a consider-
able field for investigations upon the mammalian heart in general
—an organ which has hitherto been somewhat baffling to the
physiologist.

Our experiments have all been made on dogs placed under
the influence of a full, or rather an extreme, dose of morphia—
from one to two grams of the acetate given subcutaneously in
watery solution. While this drug greatly slows the respira-
tions, and somewhat later, to a certain extent, the rate of the
heart's beat, it seems in no way to impair the vitality of this
organ ; if anything it appears rather to increase its capacity for
bearing insults—a matter deserving of further investigation. The
animal having been put very completely under the influence of
the drug, tracheotomy was performed, a cannula placed in one
carotid artery, and the pneumogastric nerve of the same side
exposed and divided so that its peripheral end was ready for
stimulation.

An incision was then made in the middle line along the
manubrium of the sternum ; the muscles, &c., were dissected
from the first pair of costal cartilages, and (the apparatus for
artificial respiration having been connected with the windpipe)
the cartilages of the first pair of ribs and the bit of sternum
between them were removed, thus laying bare the apex of the
chest cavity, which was then opened. The artificial respiration
was now stopped for a few seconds, so that the lungs might
collapse and thus expose on each side the internal mammary
artery, running along the exterior of the mediastinum and the
remnant of the thymus, to the ventral aspect of the chest wall
opposite the second costal cartilage. These arteries having been
tied, the incision along the middle line was prolonged backwards
and the skin and muscles reflected on each side so as to expose
the rib cartilages. This operation is usually accompanied by
only an inconsiderable venous oozing, after the internal mam-
mary arteries have been secured in the manner just men-
tioned.

The sternum and costal cartilages were then removed, care of course being taken not to injure the lungs. The next step is to stitch the pericardium to the chest wall in order to support the heart and prevent its receding too much when the lungs empty during expiration.

Branches of the coronary artery can now be seen through the pericardium, and a window is so cut in that membrane as to expose a branch which seems suitable, while all the rest of the heart remains protected and supported by its sac.

So far the operative procedures are tedious but present no special difficulty; but to lay bare the coronary branch and to fix the cannula in it while the heart continues to beat is much more troublesome, since any carelessness in these operations is apt so far to injure the heart as to destroy its normal beat and throw the ventricles into incoördinate fibrillar contractions, from which we have never seen them recover. The success of the attempt depends largely on the animal; in the most favorable cases the left coronary artery, after giving off its transverse branch, which runs along the auriculo-ventricular groove, passes along the septum ventriculorum on the ventral aspect of the heart, and gives off near the base of the ventricle a considerable branch to the right, which runs with a vein on each side of it, and is covered only by the visceral layer of the pericardium and some fat. Into this branch the cannula is inserted, and the blood carried by the main trunk and its remaining branches serves perfectly to keep the heart beating vigorously for several hours, as we have repeatedly found. In other cases the artery does not give off this one main branch, but (especially in large dogs) runs along the ventricles, giving off small twigs right and left which are too minute for the convenient introduction of a cannula, and are, moreover, often covered by a thin layer of the musculature of the heart in addition to the pericardium. This muscular layer adds greatly to the difficulty of successfully isolating the artery, for any wound to the proper cardiac substance about the vessels seems more fatal to the organ than anything else. Soon after such an injury it almost invariably exhibits periodic beats for a short time, and then the ventricle passes into a state of fibrillar contraction. The well-known fact that needles may be thrust into many parts of the heart without

essentially influencing its beat for a long time, inclines us to the belief that the result in the cases to which we refer is, perhaps, due to the injury of nerve trunks which may run in the heart near its arteries and which are torn with the muscle, rather than to direct injury of the muscular substance; but we have not yet had an opportunity to examine this point.

A suitable coronary branch having been found, the next step is the most difficult in the operation, viz., to tear through the visceral pericardium over the artery without opening that vessel or its accompanying veins; for the membrane is so smooth and tightly stretched that it is not easy to catch hold of; and then so tough that it is difficult to penetrate. Our method is as follows: All being ready, the pneumogastric trunk is stimulated so as to stop the heart's beat, and the artificial respiration simultaneously suspended so as to avoid movements of the heart due to contractions and expansions of the lungs. With a sharp-pointed pair of forceps the pericardium over the artery is seized and a hole torn through it by means of a needle; once this aperture is made through the tough membrane without injuring any of the vessels, the rest of the operation is comparatively easy. The stimulation of the pneumogastric is stopped and the artificial respiration resumed for a moment or two; then the heart-beat and breathing are again suspended, the edge of the hole is taken in the forceps and the membrane over the artery slit up toward the base of heart by a very fine-bladed knife. From time to time, as the heart begins to beat in spite of stimulation of the pneumogastric, the nerve is allowed to rest and the respiration is resumed, and in this way the alternate stimulation and rest are repeated as often as may be necessary in order to expose a sufficient length of the artery, to place ligatures around it, and insert a cannula in the manner adopted for any other artery. The carotid was then connected with one mercury manometer, the coronary branch with another, and, the pens being arranged so as to write exactly over one another, tracings were taken on the kymographion.

The mode of connection of the arteries with the manometers demands a word. In the first place, the three inches of the arterial end of the connecting tube between the coronary and its manometer consist of highly flexible rubber tubing. This, no

doubt, slightly modifies the pulse-waves on the tracing, but it gives to the heart free play during each beat, since the flexible tube offers no restraint, but yields readily. This soft tubing is succeeded by a glass tube, which is firmly held by a solid support, so that no locomotion of the tubing occurs beyond this point.

Movement of the bit of flexible tubing attached to the cannula does slightly alter the level of mercury in the manometer, but, as we have satisfied ourselves by careful examination, causes no feature in the tracing which can be mistaken for a pulse-wave. Beyond the piece of glass tubing mentioned above, the connecting arrangement is similar for the two arteries.

To get a true base-line, or line of no pressure, for each manometer gave us some little trouble. The base-line is often taken as that drawn by the pen when the mercury stands at the same height in both legs of the manometer, but this is seldom correct. If the end of the connecting apparatus attached to the artery be above the level of the mercury in the limb of the manometer with which it is joined, the weight of the liquid in it will affect that level, making it sink in the nearer and, of course, rise in the farther limb which bears the pen. If, on the other hand, as is more often the case, the arterial end of the connecting tube be below the level of the mercury in the gauge, the tube acts like a siphon-tube; the mercury rises somewhat in the proximal limb, and sinks to the same extent in that which carries the pen, so that in either case the base-line drawn with the two mercury columns level will be incorrect.

As we wished especially to compare the amount of arterial pressure in the coronary with that in the carotid, we had to eliminate such errors, and the more so because the manometer attached to the coronary artery was invariably above the one connected with the carotid, and so the siphon action (for the ends of the tubes farthest from the kymographion were always below the levels of the mercury in the manometers) was considerably greater. The method which we adopted gives, we think, absolutely true results. Having finished an experiment, we stopped the artificial respiration, and let the animal die of asphyxia, the manometers being meanwhile shut off from connection with the arterial system. When the animal was quite dead, and all traces of arterial pressure had disappeared, the communication with the

manometers was again opened, and the pens naturally fell with the mercury to the level which corresponded to zero arterial pressure: we, of course, satisfied ourselves that there were no clots in the apparatus. The pens were then turned away from the paper, which was next re-coiled on the drum until the beginning of the record of the experiment was reached; then, the pens being turned back again, the kymographion was started once more and each pen drew its own base line, being still connected with its artery and the position of the animal being the same as during the experiment. It has been suggested to us that the base line so obtained may not be reliable, as some arterial pressure might still remain in either the carotid or coronary vessel, or in both, after general death; but this objection we think will not :ar examination. After death from asphyxia, as is well known, e arterial system, at least in its larger trunks, is extremely mpty; a few minutes after its occurrence one may cut the aorta without the slightest spirt of blood resulting, and, indeed, even almost without bleeding at all; and the carotids, subclavians, and other large arterial trunks are obviously collapsed and empty. That under such circumstances there should be any arterial pressure possibly remaining in arteries in free and direct connection with the aorta is not conceivable.

A description of the tracings taken on the kymographion (Figs. 1—5, Pl. XXV, XXVI, XXVII) will serve best to show our results. The tracings, in fact, speak for themselves, and have been selected from a considerable number which all perfectly agree with them as to the conclusions to which they lead; we have never obtained a single contradictory record. The pulse synchronism and the similarity of the pulse-waves in the carotid and coronary under different amounts of blood-pressure and with various rates of heart-beat is remarkable throughout. In Fig. 1, Pl. XXV, we have a pulse-rate of 132 per minute, and complete synchronism in the two arteries; the mean pressure in the former being 62 mm. of Hg. and in the latter 42. The verticals, vv, cut all the tracings at points corresponding to the same instant of time. In Fig. 2, Pl. XXVI, is a tracing taken with a quicker pulse, about 172 per minute. At v', artificial respiration was stopped so as to get a dyspnœic rise of arterial pressure. As the verticals show, this does not disturb in the least the synchronism or similarity of the

pulse-waves in the two arteries. Mean pressure in coronary, 46 mm. of Hg., and in carotid 56, at the beginning, rising to 100 mm. and 120 mm. respectively just before v‴.

Fig. 3, Pl. XXVI, gives simultaneous tracings from the two arteries during extreme dyspnœa, with greatly slowed pulse and very high blood-pressure, rising in the part of the tracing given to 120 mm. of Hg. in the coronary artery and to 132 in the carotid. Ultimately the pressure rose still higher, and drove the pen attached to the coronary vessel off the top of the paper, so that a record could not be obtained. The accuracy with which each tracing reproduced the other during all the variations of pressure and pulse-rate which occurred during this observation is very remarkable, and seems to make it certain that the pressure in each artery is directly determined by the same cause, viz., aortic pressure. The contracting ventricle might conceivably increase pressure in the coronary vessels by compressing them; but variations thus produced cannot possibly be imagined as agreeing so perfectly with the variations in carotid pressure (which, on such a theory, must be differently produced and sustained) as do those given in this figure.

Unfortunately a seconds pen was not connected with the kymographion on this occasion, so that the pulse-rate cannot be stated accurately; but by taking an average from the rate of movement in other cases it may be set down, without any great error, as about 60.

In Fig. 4, Pl. XXVII, is given a tracing taken soon after the resumption of artificial respiration, which had been interrupted long enough to produce (as seen to the right of the tracing) a considerable dyspnœic rise of arterial pressure. Well marked and similar Traube's curves are seen on each tracing, and also the synchronous pulse in both arteries. This synchronism is maintained throughout all changes of cardiac rhythm and blood-pressure.

In Fig. 5, Pl. XXVII, is a tracing in which the coronary pressure is higher than the carotid (76 mm. against 64 mm. Hg.) This may perhaps be due to our having taken in this case a coronary branch nearer the main stem than usual; but it may be also, and more likely is, due to the vasomotors. The heart arteries have a very active system of these nerves, as any one who ex-

periments with them will soon observe. Not unfrequently on
laying bare a coronary branch that seemed suitable for insert-
ing the cannula we have found it apparently so small that our
endeavor seemed hopeless; and then in a minute or two it
would dilate again to at least double its previous diameter. If
it be borne in mind that the coronary branch used was always
but a small twig of the whole coronary system, it seems pos-
sible that great constriction in the rest of the branches might so
oppose the blood-flow as to raise the pressure almost up to
that in the aortic arch, and so bring it above that in the carotid.[1]
In other respects the tracing illustrates the same points as those
reproduced in the preceding figures. The heart was beating 148
per minute.

We find then that whether the heart beats slow or fast, and
whether arterial pressure be high or low, every feature of the
carotid pulse is simultaneously given in the coronary. No doubt,
with a faster-travelling roll of paper the synchronism would not
be perfect, as the carotid vessel is farther from the heart, but the
pulse-wave travels so fast that this could not be expected to be
shown on the kymograph.

There is, however, no trace of any alternation in the pulse-
waves, such as would seem necessarily to follow from an occlu-
sion of the mouths of the coronary arteries during the ventricular
systole, and such as, if it existed, the kymograph would cer-
tainly show.

The argument which was used effectively against conclusions
drawn from observations upon spirting coronary arteries, may be
brought perhaps to bear upon our work, viz., that in the earliest
stage of contraction of the ventricle, the coronary shares with
the carotid the general rise of pressure in the arterial system,
because the valve has not yet closed over its mouth; and that,
in consequence, it is to be expected that the two pens which have
travelled together during the diastole of the previous undulation

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[1] We have recently endeavored to discover the source of the vaso-constrictor
nerves of the heart, by connecting cannulæ with carotid and coronary arteries,
and then observing if a relative rise of coronary pressure could be brought about
by stimulating extrinsic cardiac nerves. So far our experiments have been con-
fined to the accelerators and have been entirely negative. We got the accelera-
tion of the pulse-rate, but no rise or fall in coronary pressure which was not
exactly duplicated on the tracing from the carotid manometer.

shall together begin their systolic journey on the new pulse-wave. This is, no doubt, quite true, and we have no objection to the argument as far as it goes. It leaves off, however, where our work begins, and does not affect the real point of the question, though it emphasizes the necessity for exact tracings which can be studied leisurely.

Since the coronary artery is freely exposed to aortic pressure during all of the diastole, and during the first fraction of the systole of the ventricle, we are not surprised to find on the tracings, at that time, complete agreement between carotid and coronary pulses; they are caused by the same thing and are therefore similar. If now we turn to the tracings described during the major portion of the systolic period, and find them duplicates one of the other, alike in form and synchronous in characters, it is hard to believe that they also are not directly dependent on the same immediate cause, *i. e.* aortic pressure. For if the valve closes as Brücke believes, the forces acting upon the two arterial contents are no longer identical; the carotid is still marking an increasing pressure due to the outflow of blood from the energetically contracting ventricle; but the coronary, cut off by the valve from influx of blood, is put under other conditions. It is not supposable that the ventricle acting upon the carotid directly through the aorta should cause it to trace a pressure curve precisely like one drawn at the same time by the coronary, upon which it is acting only indirectly (*i.e.* by raising intraventricular pressure, and so causing extra compression of the vessels in the heart substance). Nor is it conceivable that the coronary artery should have its mouth suddenly closed at one instant during the period of rising pulse-wave, and still go on tracing undisturbed a uniform rise of pressure. Under such circumstances some deformation of the coronary curve, some irregularity in the tracing, must take place.

Again, after the systole is over and the valves rebound to their position over the mouth of the aorta, a moment would come (when the period of highest carotid pressure was just past) when the coronary artery would suddenly be opened and blood would be driven into it. An injection of blood into the previously closed coronary system at this moment ought surely (even if it did not, as may be urged, raise arterial pressure in

the coronary artery, because the cardiac muscle was relaxing and making the coronary circuit easier of passage) to show itself in some break or rise, or other special feature in the pressure-changes at that moment occurring in the vessel; the tracing from the coronary vessel (now for the first time receiving blood) could not exactly agree in every respect with the tracing from the carotid artery, which is simultaneously emptying itself steadily and regularly under the force of arterial elasticity. We find, however, nowhere any indication of such a difference of events; the coronary tracing is always a duplicate of the carotid under all circumstances, and there is no sign of any periods when great circulatory changes (such as are involved in the supposition that the mouths of the coronary vessels are alternately closed and opened) are taking place in the coronary artery.

We are therefore forced to conclude that they are in the right who have maintained that the flaps of the semilunar valve are never pressed completely back against the aortic wall during systole of the ventricle. Finally we may point out that the tracings show the pressure-changes in the coronary system to be very much like those in any other branch of the aortic system—the carotid for example. It may be added in conclusion that though forced to differ from Brücke, in regard to any interference of the semilunar valve with the circulation in the coronary system, our observations in no way contradict his teaching that during ventricular diastole blood flowing into the coronary arteries aids in distending the flaccid heart. This is probably true. The complete *"Selbststeuerung"* is, however, no longer tenable; the arteries of the heart are not emptied during the ventricular diastole, so as to diminish the resistance to cardiac contraction, but are at that time always tensely filled. Moreover, as our tracings show, the little increment of pressure during the systole of a single beat, when compared with the entire mean pressure constantly at work in the coronary system, is so small that not much would be gained by blocking the mouths of the arteries in order to avoid it.

THE INFLUENCE OF DIGITALINE ON THE WORK DONE BY THE HEART OF THE SLIDER TERRAPIN, (*Pseudemys rugosa, Shaw.*) By H. H. DONALDSON, A. B., *Fellow of the Johns Hopkins University*, and MACTIER WARFIELD, A. B.

The experiments described in this paper were undertaken as a preliminary to an examination of the action of digitaline upon the isolated heart of a mammal. On examining the literature of the subject we found so much confusion and contradiction, and so frequently methods of experimentation which seemed open to objection, that we concluded it better to investigate afresh the action of the drug on the isolated heart of a cold-blooded animal before proceeding to study its influence upon the heart of the dog.

Though observations were made on the pulse-rate, arterial pressure, and the changes in the form and size of the heart, we wish now to develope only our results on the variations in the work done under digitaline, and shall therefore only make use of the above observations when they bear on that question.

It is quite agreed that digitaline has the same action on the heart whether that organ be isolated from the central nervous system or not. [1] [2] [3]

In mammals with the central nervous system intact, moderate doses of digitaline are observed to cause a rise in mean blood-pressure, which persists during the slow pulse. [3] [4] [5] [6] [7] [8]

Winogradoff [9] states on the other hand that the mean blood-pressure found in dogs is not noticeably modified by moderate doses of digitaline.

A contraction of the arterioles in the web and mesentery of the frog and the mesentery of the rabbit under digitaline has been observed by many [3] [7] [10] [11] [12]; others, however, have failed to find it.

Brunton and Meyer [13] obtained curves from a dog under morphia, which led them to maintain that the rise of pressure was due solely to the narrowing of the arterioles.

The suggestion having been made that this narrowing of the arterioles was one cause, at least, of the rise in arterial pressure, experiments were undertaken to test the point.

The results have been by no means concordant. Von Bezold[14] cut the cord in an animal showing high blood-pressure under digitaline. The pressure at once fell markedly, but it was yet a question whether it was as low as it would have been without digitaline.

To answer this it was necessary first to sever the cord and then inject the digitaline. Traube[4] crushed the cervical cord and could get no rise of pressure by the subsequent injection of digitaline.

Böhm using the same method on rabbits obtained the same results. But when, in an animal without its brain and spinal cord, he first ligatured the thoracic aorta above its inferior branches, and then injected digitaline, he obtained a decided rise of pressure.[15]

Having thus cut off much of the arterial system he interpreted the rise in pressure observed, not as a narrowing of the arterioles, but as an increase in the work done by the heart.

Görz[16] found after section of the cord a slight increase in pressure under a subsequent dose of digitaline. This rise he also attributed to an increase in work.

Ackermann[12] also states that he has often cut the cord and then found a decided rise of pressure to follow digitaline.

Attention was then directed to the heart. In 1872 Böhm[2] published an extensive article on the physiological action of digitaline. As has been mentioned, he could get in rabbits no rise of pressure after section of the cord. He argues, however, in this case, that the work done by the heart might have increased, and yet the extreme relaxation of the vessels prevented its expression as a rise of pressure. Moreover, he failed to observe a decisive narrowing of the arterioles in the frog, and, finally, direct experiments on the isolated heart of the frog led him to conclude that the work under digitaline was increased by moderate doses (.0005–.001 grm.), and decreased by large ones.

He used a " Ludwig-Coats " apparatus for feeding the heart and the formulæ of Blasius[17] for estimating the work. His cannulas were tied in the vena cava and bulbus aortæ. Placing

the arterial cannula in the bulbus almost certainly interfered
with the valves and thus introduced an important modification
into the circulation. The distance through which the blood was
raised varied in different experiments between 6 and 34 cm.
He does not state, however, his venous pressures, nor precisely
his doses of digitaline. Experimenting thus he obtained under
moderate doses of digitaline, an increase of work, lasting in one
case 23 minutes; coincident with a slower pulse-rate. The work
then decreased and never again reached its original amount.
Each experiment occupied about an hour.

When, however, so high an arterial pressure was used that
the work began to decrease, digitaline was unable to prevent the
decrease. His final opinion as deduced from his investigations
is as follows:

"Jedenfalls aber glaube ich durch die zuletzt mitgetheilten
Versuche bewiesen zu haben dass die bei der Digitalinwirkung
beobachtete Blutdrucksteigerung auf direckte Vemehrung der
vom Herzen geleisteten Arbeit zurückzuführen ist."

In 1880 Williams[18] published some investigations on the
rise of pressure under digitaline. He finds, as did Böhm,[2] that
the drug is incapable of increasing the maximal pressure against
which the heart can work. Moreover, he gets no evidence of
the narrowing of the arterioles under digitaline.

He observes that when the heart is working against a higher
pressure it undergoes a greater diastolic expansion, venous pres-
sure remaining the same, and does more work.

Digitaline he thinks affects the heart muscle like high pres-
sure, and then causes a rise in mean pressure through a variation
in the extensibility of the heart muscle. He made no direct
measurements of work.

This review of the literature indicates that further investiga-
tion of the question of work was not entirely superfluous.

The experiments which we have to record were made in the
following way.

Apparatus.

This was designed to keep the pressures (both arterial and
venous) and the temperature always constant, at the same
time to record the form and rate of the pulse, allow the estima-

tion of the work done, and permit direct observation of the form
and movements of the heart.

The venous reservoirs consisted of three flasks, arranged as
Mariotte's bottles, each holding about 400 cc. The flow from
each flask was through a rubber tube. By the use of two Y
pieces, and two other bits of tubing, the three tubes are combined
so that the blood from all the flasks flows finally through one tube,
which is connected with the venous cannula. The flow from any
flask can be stopped by a clamp. In experimenting, the middle
flask, graduated for every hundred cc., always held the blood
which contained digitaline. The others held what we call "good"
blood, to distinguish it from the above; only one flask was used
at any time. We had three pairs of cannulas varying in diame-
ter—the difference in the size of the terrapins making it impos-
sible to always use the same cannulas.

In order to reach the venous cannula the common inflow tube
passed through the end of a box. This was mainly of wood,
with a glass top. It always contained a thermometer. In this
box the animal rested firmly on its back. It was thus protected
from draughts, too rapid evaporation, and mechanical injury, and
yet always readily observable.

From the end of the box opposite the inflow passed the out-
flow tube. This was a tube of stiff rubber. First connected
with the arterial cannula, it then passed through the end of the
box, and at once branched.

One branch ended in a bit of glass tubing which was fastened
in a clamp that moved on an upright, and could thus be fixed at
any desired height; this was the outflow. The blood as it was
pumped out was caught in small beakers for a known time and
then measured; this gave us the means of estimating the work.
The other branch continued for about thirty cm. when it again
divided, one branch in this case being connected with a pressure
bottle filled with .75 per cent. salt solution, and the other joined
a manometer. In our earlier experiments a small mercury
manometer was used, but in all the later experiments a water
manometer, as described by Howell and Warfield,[19] was preferred.
The manometer wrote on the smoked paper of a revolving drum.
Time was marked by an electric pen connected with a clock
beating seconds.

The apparatus being ready, a terrapin was weighed, a cord tied tightly about the neck, the head cut off, the plastron removed, and the pericardium opened. The animal was now placed for a moment in the box, and the height of the heart above the table measured; then, while the operation was being completed, one of us arranged the inflow and outflow of the apparatus to give the desired pressures.

With the least possible handling all the vessels of the heart except two were then tied. The two generally used were the right aorta and the left vena cava superior. The left aorta and the vena cava inferior were, however, sometimes taken. When the cannulas were secured, one hundred cc. of pure defibrinated blood were sent through the heart, in order to wash out the contained blood which was liable to clot. Inflow and outflow were then clamped, the animal pithed and put in the box. The inflow tube through which blood from one flask was running was slipped over the venous cannula, while the arterial cannula was connected with the outflow. The circulation was thus established.

For feeding the heart we used fresh defibrinated calf's or sheep's blood mixed with its own volume of .75 per cent. salt solution. This mixture we designate " good blood." When digitaline is added to it, " poisoned blood."

The terrapins weighed between 437 grams and 1785 grams. Initial temperature was between 13°–21°C., with a maximum variation of 4°C. during an experiment.

The venous pressure varied between 2.7 cm. and 7 cm. The arterial pressure was always 20 cm. of the blood circulated through the heart. The feeding flasks sent through the medium-sized venous cannula, under 3 cm. pressure, 1 cc. in 1 sec. when the cannula was disconnected from the heart. The digitaline used was prepared by Merck. It was amorphous, and gave a slightly turbid solution with water or .75 per cent. salt solution. The amounts administered were from .00035 gram to .005 gram in 100 cc. of the diluted blood.

The digitaline was first dissolved—.001 gram in 1 cc. of water or .75 per cent. salt solution, and then added with a pipette to the flask half filled with diluted blood. The rest of the blood was then poured in, and thus a fairly even mixture was secured.

The time during which each observation lasted varied from 4 to 11 hours.

There is one unevenness in the apparatus to be mentioned: When one of the flasks is to be refilled with blood its outflow tube is clamped; two tubes running through the cork are then unclamped, and through one the blood is poured, while the air escapes through the other. The flask being filled, the two tubes are again clamped.

During this operation the entire contents of the flask have been under atmospheric pressure, and the liquid in the air tube has risen to the level of the liquid outside of it. The flask, when next connected with the heart, accordingly does not act at once as a Mariotte's bottle; it only becomes so when the air tube is clear of liquid: hence the initial pressure when a new flask is turned on is, for a few seconds, higher than it should be. This higher venous pressure causes a slight increase in work for some seconds. This error exists in our results, but it is practically too slight to be important.

The heart having been placed in the apparatus, was allowed to run until it did fairly even work per min. for half an hour or more. It was soon found, however, that it was necessary not only that the heart should do even work, but also that the work should be near the normal amount, because if a heart which under good blood was only pumping 2 cc., while later, under the same conditions, it showed itself able to pump 10 cc. in the same time, was in the first instance treated with digitaline, there was an increase in work independent of the drug.

Having noted this fact in some earlier experiments, we always waited until both the amount and regularity of the work done showed that the heart was acting normally.

A tracing of the pulse was usually taken for one minute every time the blood was measured.

As soon as the heart was working properly, good blood was turned off and the poisoned blood allowed to run until the quantity in the flask had decreased 100 cc. The time taken for this was noted. Good blood was then turned on again, and the attempt made to restore the heart to its previous condition.

If this was successful, the same operation was repeated until either it was impossible to recover the heart or repetition was deemed superfluous. This method, which allows of several observations on the same heart, was suggested by Prof. Martin, and is very satisfactory. In the earlier experiments we measured the blood and took a tracing once in five minutes. In the later ones, however, this was done only once in fifteen minutes—except when the poisoned blood was running through, when the observations were more frequent.

The poisoned blood which had once circulated and that which immediately followed it was always thrown away. The typical effect of a moderate dose of digitaline given in this way was primarily a slight acceleration of pulse joined with a sudden decrease in work. Soon after the flow of poisoned blood had ceased the pulse became normal; and then the work increased more slowly until the heart was doing, after an hour, for instance, as much or more work than it had previously done. The second dose appeared usually to take effect somewhat more quickly than the first, but it was not until the third or fourth dose that a slowing of the pulse usually became evident. As the number of doses increased it became in most cases more difficult to recover the heart.

Results.

We made fourteen series of experiments. Of these five must be discarded; two because of accidents during the observations; one because the three flasks did not give the same pressures; one because the blood was stale, and the fifth because the pressures were varied during the experiments. We have then left nine series, comprising thirty-four observations.

In order to express concisely what happens we have condensed our observations in the following way: Taking the total number of cubic centimeters pumped around in the 15 min. immediately preceding the giving of the poisoned blood, we found the average number of cubic centimeters per minute during that time. That number is our standard for the given experiment.

Now, the time being observed which it takes for 100 cc. of the poisoned blood to pass through the heart, the number of cubic centimeters pumped per minute for this period is calcu-

lated; when the poisoned blood is turned off and the good blood on, the number of cubic centimeters for the first 30 minutes is averaged, and the amount per minute found.

As the pressures in each experiment are constant, we can compare the number of cubic centimeters per min. in the different observations of the same series with one another just as well as the absolute work. This we have done. Taking then one amount pumped out, expressed in cc. per min., as a starting point, we look to see how the amounts pumped in the same time during the two subsequent periods compare with it. For brevity we will call the three periods mentioned "before digitaline," "during digitaline," "after digitaline."

Out of the thirty-four cases there are twenty-four in which the work "during" is less than that "before," and the work "after" less than that "during" digitaline. That is, where the work has remained decreased for at least half an hour after the digitaline; but after that time, the heart being steadily fed with good blood, has reached or nearly reached its original amount. Of the remaining ten cases there are six in which the work is less "during" than "before," but rises in the period "after" above what it was "during" digitaline. There are two in which the rise "after" goes above what the work was "before" digitaline (Series 3, No. 2; Series 7, No. 2).

In one of the remaining cases there is a slight and unaccountable rise "during" above the work "before" digitaline (Series 17, No. 1), while in one case the work increases from the first to last period (Series 7, No. 1). The number of cases in which less work is done "after" digitaline than "before" is then thirty-one out of thirty-four. This leaves us three contradictory cases to be explained.

The two exceptions in Series 7 (Nos. 1, 2) are cases in which the experiments were made when the heart, though pumping evenly, was doing an abnormally small amount before the administration of the digitaline, and it was not till something near the normal work was done that digitaline produced its usual effect. The third case (Series 3, No. 2) was plainly a case where time enough had not been allowed for recovery.

We conclude from these observations: 1. That where the heart is doing normal work the influence of digitaline is to

decrease that work; 2. That there is a rough relation be-
tween the size of the dose and the extent of the decrease; 3. It
is further to be observed that with small doses of digitaline the
pulse-rate is at first increased.

This observation has a two-fold significance. It confirms
those of Jörg,[20] Saunders,[21] Hutchinson,[22] and others, and at the
same time is a good indication that our doses were moderate.

An almost constant appearance under moderate doses was a
shrivelling of the auricles. This tendency, at first slight, became
at the end of a series of moderate doses very marked. With
a heavy dose the auricles became of course much distended.

During the period of accelerated or unaltered pulse rate the
volume of the ventricle appeared somewhat decreased, while
during the slow pulse it was plainly increased.

The question of dosage is one important in these experi-
ments. The dose is primarily the amount of the drug used.
But beyond that, the percentage in which it exists in the blood,
the length of time the heart is exposed to the poisoned blood,
and the surface of the heart acted on, are of the greatest import-
ance. For instance, our tables show (Series 8 and 11) that in the
course of an experiment much more digitaline can be given than
can be borne in a single dose. Indeed, in one series, not pub-
ished because vitiated by an accident, 10 doses of .0005 grm.
were given to a heart without any perceptible effect. This has
a bearing on the once held theory of cumulation of digitaline.
If it accumulated in the heart muscle one would expect, first, a
decided effect from numerous small doses, and second, a rather
tardy action of large ones. Neither occurs. The large doses act
with great rapidity, while the small ones produce no effect pro-
portionate to their number.

Still, it makes a difference how long the poisoned blood
remains in the heart. If two hearts are taken, one pumping
100 cc. in 5 minutes, and the other the same in 10 minutes, and
the same weight of digitaline given in the same amount of blood,
the effects will be much more marked in the latter than in the
former case.

Finally, as the heart increases in size its capacity increases in three dimensions, while the surface exposed increases only in two; thus the larger the heart the less, proportionately, the surface exposed to the poisoned blood.   All these points are worth consideration when the true dose is to be estimated.

It remains now to offer an explanation for those results which are at variance with our own, namely, the direct results of Böhm and the indirect ones of Williams.   Roy[23] has shown that the curve of extensibility of the ventricular muscle is an hyperbola.   In the case of the frog's ventricle it makes a sharp bend at about 10 cm. of water pressure, and beyond that increase of pressure produces little distension.   In the ventricle of a frog under a moderate dose of digitaline the elasticity is quite perfect, but the distensibility is noticeably increased, or if it were represented graphically, the new curve would fall even more nearly parallel to the axis of ordinates and to a much greater distance before bending than does the old one.   It is easy to see, then, that for a time the curves would not differ much.   That is, for moderate pressures the much increased capability for distension caused by digitaline would not be brought into play, but as soon as we make the pressure more than moderate, as both Böhm and Williams did, this new factor is brought in.   The distensions for equal increments of pressure are now much beyond the normal, the elasticity remains quite perfect, and the heart then does a much increased amount of work.

The fact that when the heart is working against a maximum pressure digitaline does not improve it, favors this view.   If the strength of the systole or perfection of elasticity were improved by it, then we should get an increase in work; but the heart being already fully distended, and the tendency of digitaline being to increase extensibility, it is here superfluous, and the work decreases in spite of the drug.

Thus it is plain that one important action of the drug is to increase the distensibility of the heart muscle.

Following are the condensed records of our experiments given in tabular form.

The table is constructed as follows:

At the beginning of each experiment are the most important data: Time of observation—the weight of the terrapin—position of the cannulas—the pressures used—and the temperature with its variation.

Beyond these are eleven columns of figures. Column one gives the number of the observation.

Column two: Gives in minutes and seconds the time which it took the poisoned blood to pass through the heart.

Column three: The number of cubic centimeters of that blood.

Column four: The absolute weight of digitaline given.

Column five: The proportional weight in one hundred cubic centimeters of blood.

Column six: The average number of cubic centimeters of blood pumped by the heart each minute for fifteen minutes before the digitaline was given.

Column seven: The corresponding average pulse-rate.

Column eight: The average number of cubic centimeters of blood pumped by the heart each minute while the poisoned blood was running through.

Column nine: Corresponding average pulse-rate.

Column ten: The average number of cubic centimeters of blood pumped by the heart each minute for thirty minutes after the poisoned blood.

Column eleven: The corresponding average pulse-rate.

I. No. of Observations.	II. Time.	III. No. of cc.	IV. Absolute Dose.	V. Amount in 100 cc.	VI. Av. cc. before.	VII. Pulse-rate.	VIII. Av. cc. during.	IX. Pulse-rate.	X. Av. cc. after.	XI. Pulse-rate.
1	8	40	.0003	.00075	16.	19.9	3.6	30.	5.9	21.7
2	5	39	.0005	.00125	6.8	21.5	5.4	22.	7.6	29.6
3	30	206	.0025	.0025	9.8	21.	5.4	25.	4.4	21.
4	22	100	.00375	.00375	11.2	25.	2.2	28.		0
1	25	10	.00012	.00012	8.	32.	not taken	not taken	1.5	30.
2	19	7½	.0009	.0012	3.6	32.	3.2	35.	1.9	30.
3	9	21	.00105	.0012	6.5	34.	1.1	18.	1.1	16.6
4	3	41	.0025	.2500	7.3	30.	0		0	0
1	17	175	.00175	.001	11.2	27.	11.1	27.5	7.4	30.
2	13	124	.00124	.001	12.8	32.	10.4	29.5	7.0	28.5
3	15	121	.00121	.0011	9.9	33.	8.5	28.	6.2	23.4
4	9	109	.00035	.00032	13.4	25.	12.8	25.5	11.1	19.2
1	45	100	.001	.....	1.6	38.	1.8	31.	4.7	21.
2	22	100	.001	.....	4.7	32.	4.0	16.	7.0	22.2
3	17	100	.001	.....	12.7	34.	7.0	25.7	4.9	22.5
4	8	100	.001	.....	13.3	19.	0	22.	12.6	20.5
1	6	100	.002	.....	17.7	23.	14.5	24.5	13.8	23.5
2	10	100	.002	.....	15.3	21.6	10.1	18.	8.7	30.2
3	13	100	.002	.....	12.3	14.	10.	14.5	8.3	16.
4	8	100	.002	.....	10.7	11.	8.	12.	5.5	9.5
1	14	100	.004	.....	18.6	32.	6.3	22.	10.5	16.8
2	7	100	.004	.....	11.	19.	11.3	17.	10.9	7.6
3	13	100	.004	.....	11.6	7.3	8.4	8.	1.4	2.
1	15	25	.00125	.005	19.5	29.	4.	13.		::
2	12	8	.0004	.005	2.	4.5	0.6	....		
1	4′ 30″	100	.001	.....	28.6	35.	29.4	25.	29.9	28.
2	4′ 90″	100	.001	.....	25.5	36.	22.2	29.5	21.1	35.
3	15′ 6″	100	.001	.....	18.3	29.	6.6	30.	9.3	16.5
4	8	100	.001	.....	17	25.	16.6	22	16.8	20.
1	3′ 45″	100	.001	.....	21.4	21.5	23.6	22.	15.2	22.4
2	5′ 65″	100	.001	.....	20.	22.3	16.8	23.5	13.8	35.5
3	6′ 15″	100	.001	.....	20.	22.5	11.4	24.7	1.5	20.7
4	7	100	.001	.....	20.5	22.8	16.4	12.6	11.2	18.
5	15	80	.0018	.005	18.6	25.3	6.	....	0	0

Series 3. Jan. 28, '82. Observations continued 5 hrs. Cannulas in R. Aorta and Left V. Cava Sup. V. P. 7 cm. Art. P. 30 cm. Temp. 16°-18.2°C.

Series 4. Feb. 1, '82. Weight 1019 grms. Observations continued 10 hrs. Cannulas in R. Aorta and Left V. Cava Sup. V. P. 3.2 cm. Art. P. 30 cm. Temp. 20.3°-21.5°C.

Series 6. Feb. 9, '82. Weight 437 grms. Observations continued 8½ hrs. Cannulas in R. Aorta and Left V. Cava Sup. V. P. 2.7 cm. Art. P. 30 cm. Temp. 18.5°-19.5°C.

Series 7. Feb. 17, '82. Weight 1112 grms. Observations continued 9 hrs. Cannulas in R. Aorta and Inf. V. Cava. V. P. 4 cm. Art. P. 30 cm. Temp. 19.5°-21.8°C.

Series 8. Feb. 22, '82. Weight 810 grms. Observations continued 9 hrs. Cannulas in R. Aorta and Inf. V. Cava. V. P. 3.2 cm. Art. P. 20 cm. Temp. 1?°. 15°-18°C.

Series 10. March 4, '82. Weight 1054 grms. Observations continued 5 hrs. Cannulas in R. Aorta and Inf. V. Cava. V. P. 3 cm. Art. P. 20 cm. Temp. 20°-21°C.

Series 11. March 15, '82. Weight 1280 grms. Observations continued 4 hrs. Cannulas in R. Aorta and Left V. Cava Sup. V. P. 4 cm. Art. P. 30 cm. Temp. 22.5°-25°C.

Series 15. March 29, '82. Weight 1725 grms. Observations continued 10 hrs. Cannulas in L. Aorta and L. V. Cava Sup. V. P. 4 cm. Art. P. 30 cm. Temp. 19.5°-21.8°C.

Series 17. April 6, '82. Weight 1785 grms. Observations continued 11 hrs. Cannulas in L. Aorta and Left V. Cava Sup. V. P. 4 cm. Arterial P. 30 cm. Temp. 19.2°-21.6°C.

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ON A NEW FORM OF PILIDIUM. By E. B. WILSON, Ph. B. With Plate XXVIII.

Among the many rare and interesting forms of pelagic animals taken with the dipping net at Beaufort, N. C., during the summer of 1880, were two specimens of a Nemertine larva, which, though belonging to the *Pilidium* group, is very unlike any of the species which have hitherto been described. It is a peculiar and highly specialized representative of this larval type; and though the scarcity of material prevented any careful histological study of the creature, it is, perhaps, worth while to describe it in order to point out its relations to some other larvæ of the same group.

The full-grown larva (Fig. 1) is helmet-shaped, but the upper or convex side is much more elevated than in most other species. At the summit of the bell is a rather small flagellum. The anterior margin of the bell is produced into four short blunt arms or lobes, of which two are seen in the figure. Behind these is a deep sinus in each lateral margin followed by two lateral arms on each side. The anterior of these, marked *a* in the figure, is considerably the largest of all the arms; in the position most commonly assumed it is bent backwards, so as to assume roughly the form of a sickle. All the lobes are very contractile, and the appearance of the margin of the bell varies greatly according to the state of contraction. The walls of the bell are also contractile, and the entire margin is sometimes drawn up so as nearly to close the opening. The cavity of the bell, indicated in the figure by a faint curved line, is evenly rounded, and of great size as compared with the corresponding cavity in other species. Behind and below the bell terminates in a blunt point.

The bell is of glass-like transparency, and is covered with a beautiful pavement of large epithelial cells. Scattered at intervals among these cells are small highly refracting spherical bodies which have the general appearance of oil-globules; they are much less numerous than the cells, and are not therefore to be

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confounded with the nuclei of the latter. Both the outer and inner surfaces of the bell are covered with cilia, which are short over the general surface, but become much longer and more powerful along the margins of the lateral lobes. By the action of these cilia the larva swims slowly and gracefully through the water, at the same time revolving upon an axis passing through the base of the flagellum and the centre of the lower surface. In one specimen there was an accumulation of dark reddish-brown pigment on each side of the bell near the base of the anterior lateral lobe; the other specimen was destitute of pigment.

The young Nemertines in both larvæ were fully developed, already exhibited some power of contractility, and within eighteen hours after the stage figured, abandoned the *Pilidium* envelope. They were very opaque and granular, showing very conspicuously and definitely through the transparent walls of the bell. Of their internal structure while within the larval envelope little could be made out, save the ciliation of the alimentary canal, which was rendered evident through the rapid rotary movements of the contents of the stomach. The young worm lies in the lower and posterior part of the larval envelope doubled up in a peculiar way, so that the middle and anterior (?) part of the body lies horizontally and transversely, while the remaining part projects nearly vertically upwards. The recently escaped young Nemertine (Fig. 2) is very contractile and changeable in shape, and swims with some activity by means of the fine cilia covering the surface of the body. Towards the posterior end a central more opaque mass bordered by a clearer zone could be distinguished. The creature is somewhat remarkable for presenting an appearance of distinct segmentation in the posterior part of the body. The young worms, unluckily, soon died, and it was therefore impossible to determine whether these "segments" were permanent or simply the temporary result of contraction of the body. It is highly improbable, in either case, that the apparent segments are true somites.

For some time after the escape of the Nemertine the cast-off larval envelope exhibited a striking, though deceptive, appearance of continuing an independent existence. Portions of the bell still performed well-marked contractile movements, and the organism, through shrunken and distorted (Fig. 3), still swam

with considerable energy by the continued activity of its cilia. These movements gradually ceased, however, and the remnant of the *Pilidium* at length died.

This larva, which for the sake of convenience we may call *Pilidium brachiatum*, is of interest, so far as its general features are concerned, in two respects, viz. in the highly specialized nature of the marginal lobes, and in the great relative size of the larval envelope. Fig. 4 represents a species of *Pilidium* occasionally found in the southern Chesapeake, which is closely similar to the common European *P. gyrans.* In this species the bell has a very different form from that of *P. brachiatum*, the apical flagellum is much larger, and there is only a single marginal lobe on each side, which is, however, very large. The young worm, which is shaded in the drawing, occupies a different position in the larva, and the bell has scarcely any cavity. A comparison of this form with *Pilidium brachiatum* shows that the larval envelope in the latter species is proportionally three times as large, at least, as in *P. gyrans ;* and the striking dissimilarity of the marginal lobes in the two forms shows how differently the tracts of locomotor cilia have been modified to increase their extent and efficiency. If we extend our comparison to other species of *Pilidium* we find a rather interesting series of modifications in the marginal lobes, to illustrate which I have introduced figures of three European species, viz. *P. auriculatum* (Fig. 6), after Leuckart and Pagenstecher, and two species (Figs. 5 and 7) after Metschnikoff. It is clear that the marginal lobes in these three species, although of different forms, correspond with each other and with those of *P. gyrans* (Fig. 4). In each case, however, the lobe has acquired a character of its own; this is especially marked in Fig. 5, where the margin of the lobe is crenate and the cilia are disposed in definite tufts separated by bare spaces. In *P. auriculatum* the marginal lobe assumes nearly the same form as the antero-lateral arm of *P. brachiatum* (Fig. 1, *a*), and the two appear to be homologous. A comparison with Fig. 7 strengthens this conclusion. The lateral lobe has here assumed a slightly different form, being intermediate between those of *P. auriculatum* and *P. gyrans*, but the anterior margin is produced into two slight prominences on each side which correspond in position with the anterior arms of

P. brachiatum. The outline of the bell, also, is to some extent intermediate between the high-arched form of *P. brachiatum* and the more flattened expanded form of *P. gyrans.* Thus the form of this species (Fig. 7) shows clearly how the highly modified *P. brachiatum* may have been derived from the common type represented by *P. gyrans,* or how both may have arisen from a common form. Attention may be drawn also to the great variation in the size of the flagellum in the various species; in some cases it may even be replaced by a tuft of long cilia.

We are thus enabled in *Pilidium* to trace out in some detail certain modifications which are due entirely to adaptation to larval life, and which do not stand in any sort of relation to the conditions of adult life. It is somewhat remarkable to find the tract of locomotor cilia so variously modified; for the conditions of larval life, so far as locomotion is concerned, seem to be much the same for all the species of *Pilidium.* The larvæ would seem to be capable of ready modification through the action of causes apparently insignificant, or else the conditions affecting the life of such a pelagic creature are more varied than appears at first sight.

The rotation of the larva upon its vertical axis, which is characteristic also of other species of *Pilidium,* is worth noting on account of the significance which Rabl has ascribed to this movement in his well-known "Blastæa Theory" (Entwickelung der Tellerschnecke, Morphologisches Jahrbuch, Vol. V, 1879). The larvæ of many Cœlenterates have been observed to perform movements of rotation; and from this circumstance has resulted, according to Rabl's theory, the acquisition of a radiate structure not only in the larva but also in the adult. If, on the other hand, the movement be unaccompanied by rotation, if it be linear and not spiral, then, according to the theory, the tendency will be towards the development of a bilateral instead of a radial symmetry. Rabl's theory, it is true, considers especially the movements and symmetry of the ancestral "Blastæa," from which the Cœlenterata and Bilateralia have been derived; but his argument from the free-swimming larvæ of Cœlenterates is based on the assumption that the particular form of symmetry shown in these larvæ stands in causal relation with their mode of locomotion. In *Pilidium* is found, contrary to the demands

of the theory, a strict and pronounced bilateral symmetry co-
existing with a spiral movement, and the same is true of many
other larvæ, as, for instance, among the Chætopod annelides.
And further, some Cœlenterate larvæ—*e. g.*, that of *Renilla*—
which perform marked spiral movements, are, to say the least,
as much bilateral as radiate. Hence, it seems probable either
that Rabl has attributed too much importance to the character
of the movements of the primitive Blastæa, or that the argument
drawn from the locomotion of existing larvæ cannot be sustained.

EXPLANATION OF PLATE.

Figure 1.—*Pilidium brachiatum*, nov. sp., from Beaufort, N. C.;
from left side. × 60.

Figure 2.—The same; young Nemertine soon after its escape from
the larval envelope, × 120.

Figure 3.—The same; larval envelope which has been cast off.

Figure 4.—*Pilidium* closely resembling *Pilidium gyrans*; from
the southern Chesapeake, × 70.

Figure 5.—*Pilidium* with peculiarly modified marginal lobes;
after Metschnikoff.

Figure 6.—*Pilidium auriculatum*; after Leuckart and Pagen-
stecher.

Figure 7.—*Pilidium*, sp.; after Metschnikoff.

ON THE POLAR EFFECTS UPON NERVES OF WEAK INDUCTION CURRENTS.—By HENRY SEWALL, Ph. D.

More than a year ago I was engaged at Leipzig, in company with Prof. v. Kries, in studying the action of two successive sub-maximal stimuli upon each other in curarized muscle.

The results then obtained appeared to the authors, at least, to recommend the simplicity of the methods employed; and they were accordingly used subsequently in a large number of experiments performed with a view to discovering the physiological interaction of rapidly succeeding stimuli applied indirectly to the muscle through its nerve. It was soon evident, however, that the method was quite inadequate to the task proposed, and that portion of the work was for a time abandoned; but not until it was clear that the difficulty experienced was due to the specific action of the electrical currents upon the nerve.

It is proposed in the present paper to consider the influence on the nerve of very weak induction currents passing through one pair of electrodes, as shown by their effect upon submaximal muscular contractions excited through a separate pair of electrodes.

The records were taken by means of an elaborate form of pendulum myographion described·in another place.[1] The recording lever was very light, and magnified the contractions some eight times. The weight hung upon the axis of the lever. The muscle used was the gastrocnemius of the frog with its attached nerve. The tissues were freed from blood before excision by a stream of 0.6 per cent. NaCl through the aorta. The experiments were made upon several different species of frog, and occupied the month of February and part of May. Two du Bois induction coils, without the iron cores, placed at right angles to each other and several feet apart, supplied each pair of electrodes upon which the nerve rested. The nerve was usually laid between two strips of moist filter-paper upon platinum wires, which were

[1] Journal of Physiology, Vol. II, p. 164.

stretched over an ebonite block, and this was then covered to prevent evaporation. In this case no other moist chamber was used, and the muscle was simply inclosed by the skin. Occasionally the nerve was placed upon platinum wires without being inclosed in moist paper. In such instances the electrode pairs had to be moved much nearer together than previously, in order to obtain the results to be described. A modified form of the usual nonpolarizable electrode was also employed, which has been found useful both in this and in general laboratory work. Four glass U tubes were cemented, each by one limb, into holes bored into an ordinary microscope slide. Clay plugs filled the cemented limbs, and the amalgamated zinc wires dipped into the free ends of the tubes. With a little care the zinc sulphate solution was prevented from rising to the top of the clay. After the nerve was laid on the upper ends of the clay plugs, it was covered by a glass slide borne by narrow glass slips cemented along three sides, so as to cover in a little chamber which could be kept moist by salt solution contained in a tube fastened into the lower slide.

In the experiments, unless otherwise indicated, the two keys of the myographion were placed so as to be opened simultaneously by the swing of the pendulum. The shock from one induction apparatus was so far weakened that it just failed to call forth a contraction from the muscle, and was, therefore, for itself inefficient. The intensity of the stimulus from the second coil was regulated to excite a contraction varying from about one-tenth to three-fourths the height of a maximal contraction.

When the two pairs of electrodes are pretty far apart on the nerve, one inch or more, the results from double stimulation are not at all regular. There is good evidence of an interaction of stimuli, however far separated on the nerve, but not in the sense to be considered below. The results in such cases are too irregular, and their causes too obscure, to be treated at present.

When the electrode pairs are separated by a short distance on the nerve, resting within three-quarters of an inch of each other, the height of the contraction due to the single efficient stimulus is profoundly and regularly altered under the influence of the other stimulus (which is itself too weak to produce a contraction) when both are let simultaneously into the nerve. This interaction is more pronounced the nearer the two pairs of wires are

together. When the nerve is not covered by moistened paper, the inner or adjoining wires of the electrode pairs must be approached to within one-fourth to one-eighth of an inch of each other. When the electrode pairs are moved farther and farther apart the constancy of the results of double stimulation gradually fails in an order which has not been studied; and, usually, when the inner wires are separated by an inch or more, the effects to be immediately described do not regularly appear.

The results obtained under the conditions described may be conveniently arranged as below.

I. When the upper stimulus, that farthest from the muscle, is able by itself to produce a contraction; the lower stimulus, that nearest the muscle, taken alone being inefficient:

a. When the upper is descending and the lower descending, double stimulation gives a strong diminution of the single contraction obtained from the upper stimulus.

b. When the upper is descending and the lower is ascending, double stimulation gives a strong increase in contraction over the single.

c. When the upper is ascending and the lower ascending, double stimulation gives slight increase over the single.

d. When the upper is ascending and the lower descending, double stimulation gives diminution of the single.

II. When the upper stimulus taken alone is inefficient to produce a contraction, the lower being by itself efficient:

a. When the upper is descending and the lower descending, double stimulation gives an increase over the single contraction.

b. When the upper is descending and the lower ascending, double stimulation gives strong increase over the single.

c. When the upper is ascending and the lower ascending, double stimulation gives strong diminution of the single contraction.

d. When the upper is ascending and the lower descending, double stimulation gives a diminution of the single.

Below is a table embodying the results of one experiment made in May, with the use of platinum electrodes whose inner wires were nearly one-third of an inch apart, the nerve resting on moist paper. The numbers refer to the heights of the contraction curves measured in millimetres.

UPPER STIMULUS ALONE.				LOWER STIMULUS ALONE.				Height of contraction from double stimulation.	REMARKS.
Efficient.		Inefficient.		Efficient.		Inefficient.			Double Stimulation gives
Ascending.	Descending.	Ascending.	Descending.	Ascending.	Descending.	Ascending.	Descending.		
	3.5					0		15.5	Strong increase.
	12.						0	0.	" diminution.
10.5	..						0	1.	Diminution.
			0		4			14.5	Strong increase.
	0				15			9.5	Diminution.
			0	12				16.	Increase.
	0			14				6.	Strong diminution

It need hardly be pointed out that these results may be described as due to the polar influences of the inefficient stimulus.

The fact indicated in the preceding, that the excitation developed at the kathode of the efficient stimulus is depressed in the neighborhood of the anode of an adjoining pair of electrodes, and conversely, presents nothing essentially new; but it is interesting to observe the results in cases 3 and 5 of the table, in which it is shown that not only does an increase in the intensity of an anodic area diminish an excitation wave passing through that area from a kathode above, but even when the exciting kathode is nearest the muscle, the contraction caused by it is lessened when the intensity of the anodic phase higher up on the nerve is increased. It is seen at once that all these results follow the general conditions of what is known as the "law of contraction."

In nearly all the experiments the two stimuli were let simultaneously into the nerve. When the myographion keys were so arranged that the two shocks succeeded each other at different time intervals, whatever the order of succession, the interaction of the two stimuli gradually diminished with the increase of the interval and failed altogether when this was still very small— that is, about 0.001 second. Comparatively little attention was paid to this aspect of the work; but there was in no case evidence of an oscillation of electrotonic condition at either pole of the reacting current, such as occurs after the cessation of a galvanic current in the nerve.

It is not very clear what relation the two phenomena, the "action current" and the "electrotonic current" set up in a nerve by an induction shock, bear to each other. The evidence[1] goes to show that the two changes appear simultaneously on stimulation and progress with equal velocity. Hermann[2] is the only investigator, as far as I know, who has made a definite attempt to analyze the electrotonic phases of the induction current in this connection; and any one reading this paper must be struck with the indissoluble character of the bond uniting the purely electrotonic with the physiological excitatory changes set up in a nerve by electrical stimulation. Grünhagen,[3] starting from some results of Harless, in which the latter found that by the double stimulation of a nerve in two places he sometimes got an increase and at others a diminution of the contractions from the single stimuli, and working with constant currents, decides that two effective stimuli applied simultaneously to the extremities of a nerve summate; but if one stimulus be by itself ineffective, then in no case does it influence the effective stimulus. Grünhagen's work, however, has little in common with that detailed above. Some of the experiments of Wundt[4] touch upon isolated points of the questions considered here. A short résumé of the results of work upon the interaction of electrical stimuli in nerve given by Hermann[5] may be of use to one who is not acquainted with the literature of the subject.

[1] Helmholtz, Monatsbericht. d. Berlin. Akad. 1854, p. 329 Pflüger, Electrotonus, p. 442. Tschirjew, du Bois' Archiv, 1879, p. 525.

[2] Hermann. Pflüger's Archiv, Bd. XVIII, p. 574

[3] Zeitschr. f. Nat. Med., 3te Seite, XXVI, 1866.

[4] Wundt. Mechanik der Nerven.

[5] Hermann. Hdb. der Physiologie, Bd. II, S. 109.

It appears to the writer that a consideration of facts such as those which have been detailed must affect to a great degree the physiological significance of all results which follow the very rapid succession of stimuli in nerve muscle preparations, and if this be true the adaptability of the electrical method to such experiments is extremely doubtful.

Some interesting conclusions of Dew-Smith[1] from "double nerve stimulation" have been kept in mind throughout this work. That author found, essentially, that when a nerve was simultaneously stimulated by submaximal induction shocks at two different points, the muscular contraction ensuing did not represent an addition of the contractions from the single stimuli, as might have been expected, but about equalled the contraction which was to be obtained from the lower single stimulus, that nearest the muscle, acting alone. He suggests as an explanation that the excitation-wave passing downward from the upper pair of electrodes is "blocked" by the wave going upward from the lower electrodes and is thus practically annihilated. The suggestion was a valuable one as offering a possible clue to an explanation of the difficult question of physiological inhibition, and it seemed highly desirable to find the true meaning of the outcome of the experiments.

These results, however, appear to be readily explained when considered as a special case of the phenomena whose general relations have been considered in this article. Let us consider the effects brought about in the contractions from double stimulation when the strength of one excitation is varied. When, for example, the lower stimulus is ascending and efficient, the upper being ascending and inefficient, double stimulation gives a contration smaller than that obtained from the lower stimulus alone. Let, now, the strength of the upper stimulus be gradually increased; there comes a point where the excitation from the kathode of the upper electrodes balances the depressing effect upon the lower stimulus of the upper anode, and, as far as I have observed, this point is reached when the single contractions are not far from equal.

The resultant of the interaction of the two excitations depends, of course, altogether upon their relative strength and direction in the nerve.

[1] Dew-Smith. Journ. of Anat. and Phys., Vol. VIII. 1874, p. 74.

RESEARCHES ON THE GROWTH OF STARCH GRAINS.

By A. F. W. SCHIMPER, Ph. D.[1] With Plate XXIX.

I.

The starch grains found in many growing chlorophyl-containing plant parts, show a constant structural peculiarity; these grains, usually tablet-shaped in the observed cases, present ragged edges, sometimes perforated. The broad surfaces are very uneven, and present under the microscope a spotted appearance, produced by superficial sculpturing, and, in many cases, also by internal vacuoles. From the results of the following researches, these appearances must be ascribed to partial solution, due to the fact that some of the starch is used for the growth of the organ. This conclusion rests, on the one hand, on the fact that after the cessation or abatement of the growth of the organ concerned, the starch granules deposited do not possess the above characters; on the other hand, on the fact that similar appearances occur in germinating seeds (*e. g. Zea mais.*)

After the starch-bearing organs have ended or greatly slowed their growth, the formation of normal starch begins; usually some new spherical starch granules appear, which show no trace of the above described structure; in addition, the already present granules increase in size. *This increase does not occur, as one would expect, in the interior of the grain, but in the form of an originally very thin and gradually thickening, shiny and strongly refracting stratum, deposited around the original corroded grain.* This layer is not itself corroded, but shows, of course, prominences and pits corresponding to those of the corroded grain. The subsequently deposited strata agree in character with that first laid down, but the inequalities of the surface become gradually obscured, so that it is often smooth in a fully formed grain. In the centre of this complete grain, when

fresh, one can, however, with suitable illumination, still detect the original corroded granule.

The appearances just described may be seen in many different species of plants.

Among others, I have seen them very beautifully in the seeds of of some *Leguminosæ*. The starch grains of the cotyledons *Dolichos lablab* (Figs. 1–3), which is one of the plants most suitable for the purpose, first appear when the seeds have attained one-third of their full size. They are then flattened corpuscles, with very lumpy surface, surrounded by chlorophyl. The starch grains retain the same form and structural peculiarities, though increasing considerably in size, so long as the cotyledons are growing and possess a vivid green color. With the cessation of growth and the diminution of the chlorophyl the formation of the final " reserve " starch commences. First appear, in most cases, glistening bluish-shimmering spots on single prominences or on one side of the starch grain ; soon the whole grain is surrounded by a thin layer of dense non-corroded substance. The starch formation thenceforth proceeds uniformly. In the completed grain one clearly recognizes the corroded uneven kernel.

Starch formation in the seeds of *Vicia faba* agrees essentially with that observed in *Dolichos*. In *Phaseolus* the grains are originally spindle-shaped, and with a less uneven surface than that of the plants above named. Nevertheless, the same mode of development may be recognized in them.

In the medullary parenchyma of *Cereus speciosissimus* (Figs. 4–7), the starch formation is like that in *Dolichos*. The tops of the stems examined contained, close beneath the "punctum vegetationis," many large starch grains with smooth surface. The developmental processes which are briefly described below refer to actually growing stems, in which starch formation is easily observable. One or more starch grains arise in the chlorophyl granules accumulated around the cell nucleus. Here, also, they appear as minute angular tablets, but with a not very greatly corroded surface. With the diminution of the chlorophyl grains, which towards the last form only thin membranes around the starch grains, the definitive starch development commences, and proceeds as in *Dolichos*. Here also the primary corroded tablet is clearly recognizable in the completed grain.

There can, therefore, be no doubt that the inner parts are not, as Nägeli maintains, the youngest, and the outer the oldest; the exact opposite is the case. *The growth of a starch grain occurs by deposition on its exterior.*

More careful examination of the development of starch grains results in many other facts which are incompatible with Nägeli's theory. The starch grains of *Dieffenbachia seguina* are, for example, very instructive; [1] in contact with a second chlorophyl granule they obtain a new system of layers, deposited on the primary. In the following sections we shall meet with still other phenomena conclusively showing the untenability of Nägeli's doctrine; those described in the present section are, however, sufficient. My immediate object is to examine more closely and explain those properties of starch grains which have been regarded as proving a growth by intussusception.

These properties are generally known, and will have, moreover, to be closely discussed in the course of this article. I content myself, therefore, for the present, with briefly stating them in the order in which they are discussed in the following pages: 1. The differentiation into regions containing different amounts of water. 2. The differences in regard to percentage of water, and sometimes of shape, between the small granules and the inner strata of the larger. 3. Unlike rate of growth in different directions. 4. The mode of growth of compound and partially compound grains.

One might be inclined to assume, as Dippel[2] has for the cell membrane, that there occurs an intussusception growth of layers first deposited by apposition, but in such cases the original kernel would certainly be lost, which is by no means the case. On the other hand, we shall see that all the properties of the starch granule may be explained without the assumption of any intussusception.

So far as concerns the objection which one might raise beforehand in opposition to the whole drift of these researches, viz., that cell membranes undoubtedly grow by intussusception, and that consequently the so similar starch grains must do likewise,

[1] See Schimper. Untersuchungen über die Entstehung der Stärkekörner. Bot. Ztg., 1880. Taf. 13, Fig. 13.

[2] Die neuere Theorie über die feinere Structur der Zellhülle, etc., 1878.

it is no longer tenable, after the well-known researches of Sachs, Traube and De Vries on the influence of turgidity on the growth of the cell membrane. These researches have completely eluci- dated the surface-growth of the membrane by intussusception, since they have shown that it only occurs under the action of cell turgidity, and consists in a constantly repeated exceeding of the elasticity limit, with an immediately following deposition of solid particles in the interstices. Increase in area and increase in thickness of the cell membrane are, therefore, to be attributed to quite different causes; from the fact that the former takes place by intussusception, the conclusion is not justifiable that the latter occurs in the same method. Still less can it be applied to the starch grains where there is no question of turgidity.

II.

In Nägeli's theory the part concerned with the developmental history of the kernel and of the layers in simple starch grains is undoubtedly the best thought-out portion. The facts that the kernel consists of a soft material, while starch grains of like size in the same plant are dense; and that the outer layer is always poor in water, even during the deposition of layers con- taining different proportions of water (which necessarily would lead to an equally frequent appearance of a peripheral layer, rich in water), appear entirely inconsistent with growth by appo- sition, while they find a satisfactory explanation through the intussusception theory.

It seems desirable, before stating the results of my own re- searches, to present extracts from Nägeli's great work, giving his view of the history of the differentiation of starch grains into kernel and layers.

According to Nägeli the developmental history of a simple starch grain is as follows: [1]

"All starch grains are spherical in the earliest stage and consist of a dense material. Then in all cases a spherical kernel of softer mate- rial separates, and after it has increased in size divides again con- centrically into a new small spherical kernel, and an inner dense and outer softer stratum, the latter strata forming spherical shells

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[1] Die Stärkekörner, S. 230.

around the kernel. The process may be repeated once or several times. Less frequently a small spherical, denser kernel is deposited in the large, more watery original one. The outer stratum as well as those which have been formed by division of the kernel divide from time to time concentrically after they have attained a certain thickness. Usually one dense stratum splits into two of similar character, with an intermediate soft one : more seldom a soft stratum is divided by a denser. In addition a thickening occurs ; it may be observed in the soft strata and in the kernel even when the hard strata have attained considerable density. If, however, the strata differ from one another so little in consistency that the whole mass appears homogeneous, it is the dense parts which first appropriate more material."

Nägeli's theoretical explanation of these processes is as follows :[1]

" If we conceive the spherical beginning of a starch grain as consisting of similar concentric molecular layers, then any nutritive liquid entering will first lay down new particles in the surfaces of these layers. This results from the fact that the resistances are there less than those which would be met with on deposition between the layers. . . . . Let us assume that the molecular layers in the whole grain simultaneously and uniformly increase : then any two neighboring layers will exhibit a tendency to separate from one another, since the radius of the outer would with unimpeded growth increase more than that of the inner. Since the adhesion does not allow a separation this tendency results in a tension, positive in the outer, negative in the inner layer. Since all molecular layers in the entire grain behave similarly, the positive tension in the particles of each one must decrease from the surface to the centre and the negative tension increase. The tension in a given layer must act on the next outer layer as a contracting, on the next inner as an expanding force. In fact, however, the nutritive fluid does not nourish all molecular layers simultaneously and uniformly. Its concentration diminishes as it approaches the centre. The condition that the outer molecular layers are earlier and more richly nourished than the inner must increase the tension between them. That the outer layers have a greater tendency to expand than the inner is proved by various facts.

" So soon as the tension under which, in consequence of growth, the molecular layers find themselves has reached a certain degree they separate from one another, and new layers are deposited between them. This will occur most frequently where the tension most

[1] *Loc. cit.* S. 289.

easily overcomes the adhesive force. The adhesion is directly pro-
portional to the superficial area of the molecular layers. The tension is
primarily present as a surface force, and it is merely a question how it
is changed into a radial or separating force. Calculation shows (1)
that the radial force which holds in equilibrium a tangential or sur-
face force, in a system of spherical shells or cylindrical envelopes of
like thickness and similar property but of different size, stands in
inverse proportion to the length of the radii ; and (2) from this first
fact it results that when two spherical or cylindrical shells in contact
with one another and of like thickness and elasticity grow by like
quotients superficially, the force which tends to separate them is
inversely proportional to the square of the radius. The molecular
layers are so much the easier separated from one another as they are
nearer the centre of stratification. . . . .

"The larger the young dense grain becomes the greater becomes
the unlikeness in density and cohesion between surface and centre,
and so much the greater becomes the negative tension in the inner-
most part of the mass, and the tendency to deposit material there.
When these ratios have attained a certain value, a space filled with soft
material is rapidly formed in the central point of the grain. A
similar process occurs subsequently in the dense cortex, and later
repeatedly in the dense strata. These grow thicker : so soon as they
have attained a certain thickness the dissimilarity of tension in outer
and inner molecular layers produced by surface growth, and the
effort to separate from one another, become so considerable that it
cannot longer be met by deposition of new material of similar density.
These results, therefore, are actual separation ; a space filled with soft
material appears."

I believe that I have above given the most important points on
the theory of growth of simple starch grains. Subsequently
Nägeli endeavors to explain the occurrence of dense strata in
soft, and in the kernel ; also the condensation of soft strata
throughout their whole thickness. I have failed to completely
understand these parts of his work, and since they appear to me,
for reasons to be immediately stated, much less essential than those
dealing with the formation of the kernel and of the soft strata, I
must, so far as they are concerned, refer the reader to the original.

Some of the phenomena regarded by Nägeli as undoubted
are only assumptions facilitated or made probable by the theory,
namely (1) The occurrence of new strata in the kernel. (2) The
occurrence of new strata in the soft ones. (3) The condensation

of the soft strata throughout their whole thickness. (4) The absence of any increase in thickness in the outermost strata. With reference to these points observations are entirely wanting. They could only be established if the development of a starch grain could be directly watched, or if it was so far the same for all the grains of an organ that the comparison of specimens of different ages could give an accurate notion of the developmental history of a single grain. As is well known, neither of these alternatives is the case.

Moreover, Nägeli himself concedes, with reference to the formation of dense strata within soft, that he has made no sure observation on the point. " Like the dense strata, the soft, without doubt, also split, forming two superficial soft strata and a median denser. *However, this process is only seldom and to a partial extent to be clearly seen ;* much less frequently than the division of the dense strata, which in innumerable cases presents itself with all certainty."[1] He seems also to have seen no very clear picture of the occurrence of dense strata in the nucleus. He says rather, at the end of his description, referring to this point, " No grains were drawn which give an accurate picture of it. One can, however, form a tolerably accurate idea by the aid of Figs. 20 and 21, plate XVII."[2]

The most important of the phenomena upon which Nägeli's theory is based are, however, undoubted facts. The developmental history of a starch grain, as deduced with certainty from a comparison of specimens of different ages, is as follows: (1) The appearance of starch grains in the form of strongly refracting corpuscles, poor in water; (they are by no means always spherical, as Nägeli assumes). (2) Differentiation of the originally homogeneous grain into a central kernel, rich in water, and a peripheral dense stratum. (3) In later conditions the kernel is surrounded by three strata, of which the middle one is always rich in water ; such a layer never appears as peripheral, and it must, therefore, be formed through a cleavage of the first dense stratum. (4) The number of strata increases ; but the outer one is always dense. (5) As the starch grain increases in volume, the proportion of water in its inner parts increases.

The explanation of these appearances I find in certain long-known physical properties of starch grains, to the consideration of which I now proceed.

[1] S. 234. [2] S. 233.

The compression of a starch grain leads to the production of numerous clefts, which in a simple grain usually run in directions perpendicular to the surfaces of the strata; never parallel to them. Careful crushing of the grains under water does not as a rule split them into fragments. They appear even after the action of very strong pressure coherent, greatly flattened structures, traversed by numerous radial fissures.

The cohesion of a starch grain, therefore, varies very remarkably with the direction; it is small tangentially, very great radially. In the latter direction its substance is very extensible, while extensibility in the tangential direction seems almost entirely absent.

The formation of clefts and the flattening are not the only results when starch grains are crushed; on the contrary, the grains experience a change consisting in a more or less marked jelly-like swelling.

That mechanical means bring about the tumefaction of starch grains, has been observed by Nägeli and Schwendener.[1] According to them, the phenomenon occurs very clearly when starch grains are cut; the parts adjacent to the cut surface assume a swollen character. According to W. Nägeli[2] this swelling, which occurs whenever a starch grain is subjected to mechanical injury, is to be regarded as dependent on a slight degree of the same process which takes place when starch is boiled in water.

The phenomenon, both as regards amount of swelling and the place where it occurs, differs with the strength of the pressure exerted. Weak pressure leads only to swelling of the innermost parts of the grain. In this case the kernel appears as if considerably increased in size, since, in consequence of the pressure, the layers immediately surrounding it have become entirely like it in light-refracting power. The outer layers only swell on exposure to stronger pressure.

The swollen part contracts on drying; its light-refracting power becomes again like that of uninjured grains, except the most strongly swollen parts, which remain less refracting. A second moistening brings about renewed swelling.

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[1] Das Mikroskop. 2 Aufl. S. 433.

[2] Beiträge zur näheren Kenntniss der Stärkegruppe, S. 25. After very strong swelling organic coloring matters are imbibed in small quantity.

Mechanical actions are, therefore, capable of imparting to the water-poor parts of the starch grain the characteristic properties of the water-rich parts, namely, greater wateriness and less light-refracting power.

Could the proof be furnished that in the growth of starch grains by simple surface deposition, forces were set in play which must result in the swelling of different parts in such a way that the known differentiation of the grains would be produced, then the question as to its origin might, without doubt, be regarded as solved.

According to Nägeli, we must regard tensions as the chief active forces in the differentiation of the kernel and of the layers. These tensions, as shown by the not unfrequent presence of fissures in starch grains, may attain considerable intensity. We have to more closely examine the cause of these tensions and their possible rôle in the development of the starch grain.

That starch grains swell in water has been generally known for some time. Nägeli, however, first showed that the deposition of water did not occur in all directions, but is much greater parallel to the stratification than perpendicular to it.

Among other things this conclusion is based on the direction of the fissures that take place on drying; this is always perpendicular to the stratification. If the water were uniformly diffused in the starch grain, then clefts must occur in other directions also. The great extensibility of the swollen grains in a radial direction diminishes very considerably with loss of water, and would, therefore, oppose no hindrance to the formation of fissures.

The unequal deposition of water shows itself most clearly when one suffers the starch grain to swell strongly under the influence of acids, or potash, or heating. It then comes out in the clearest manner that the maximum water deposition is parallel to the stratification; the least, perpendicular to it. Nägeli has instituted a series of measurements on the starch grains of *Canna* and of *Curcuma zedoaria*, which indeed (since the strata are not even, but curved in an hour-glass form) only express the relations approximately, but, nevertheless, give some idea of the greatness of the difference; they may, therefore, be here repeated.

Canna.[1]

The starch grains of *Canna* have, as is known, a flattened form and very excentric kernel; most of the strata are incomplete. On swelling a deep pit is formed on the side where the kernel lies, in consequence of the preponderating extension in the cross direction. The length of grain I was measured to the bottom of the pit; that of grain II to the points of folds on each side of the hollow. In the latter, therefore, the difference given is too small.

	I.		II.	
	Length of grain.	Breadth.	Length.	Breadth.
Before swelling,	61	14	74	55.5
After swelling,	100	150	240.5	203.5
Ratio,	1 : 1.6	1 : 11	1 : 3.2	1 : 3.7
Increase per cent.,	64	971	225	267

Curcuma zedoaria.[2]

The starch grains of *Curcuma zedoaria* have, as known, essentially the same structure as those of *Canna*, and as regards swelling behave similarly.

	Length of grain.			Breadth.		
	1.	2.	3.	1.	2.	3.
Unchanged,	59	59	66	28	28	25
After swelling,	85	77	90	87	98	105
Ratio,	1 : 1.4	1 : 1.3	1 : 1.4	1 : 3.1	1 : 3.5	1 : 3
Increase per cent.,	44	31	36	211	250	200

The appearance is, however, so conspicuous that direct measurements are not necessary in order to convince oneself of the want of uniformity in the swelling. Figs. 21 and 22 show a starch grain of *Canna* before and after swelling.

Another noteworthy appearance, brought about by the preponderating swelling in the transverse direction, is the concave folding of the cut surface of starch grains which have been bisected through the kernel. One readily obtains such grains on cutting a *Canna* rhizome with a sharp razor.

The predominance of the tangential directions when compared with radial as regards water deposition, brings about tensions.

[1] *Loc. cit.* S. 76.   [2] S. 77.

If the starch grain consisted of loose molecular layers these would separate from one another when the grain swelled up; but since the layers actually cohere firmly, each layer is strained positively with reference to the one on its inner side, and negatively with reference to that on its outer side. These readily comprehensible consequences of uniform surface increase of the molecular layers, without corresponding radial increase, have been arrived at by Nägeli by means of calculation.

If the tensions have reached such intensity that the limit of elasticity is exceeded, and the layers can in consequence follow their tendency to separate, this cannot occur through the formation of fissures running parallel to the stratification, as Nägeli assumes. The earlier described appearances of compressed grains show, on the contrary, that a traction acting vertical to the layers can produce an extension, but not a tearing in that direction. The starch grains can be extended by pressure to the extent of several diameters without the formation of tangential fissures. The stretching, however, causes, as shown by the same experiments, a swelling-up of the substance, which assumes the characteristic properties of the more watery parts of the normal starch grain.

If we seek to take into account the effect of these tensions on the developing starch grain, we find that the formation of the kernel and of the soft strata actually occurs where these tensions must exhibit themselves.

The developmental history of a starch grain is, without doubt, as follows. It consists originally of homogeneous, dense material. When, in consequence of non-uniform water deposition, the increasing tensions have attained such a degree that the elasticity of the grain can no longer resist them, the material in the centre of the grain must be extended and brought into a condition of greater swelling and less light-refracting power. Observation, in fact, shows that when a starch grain has exceeded a certain size, a less refracting strongly swollen spot, the kernel, appears in its centre.[1] The central formation of the kernel depends, as Nägeli has proved by calculation, on the fact that action of the tensions is there most powerfully exhibited. As regards this point it

[1] Compare the representation of the formation of the nucleus, as given by Nägeli, *l. c.* S. 309.

naturally amounts to the same thing whether the tensions, as Nägeli assumes, depend on an uneven deposition of starch molecules, or, as I (basing my belief on observation) contend, upon a non-uniform deposition of water molecules.

The formation of the kernel causes, of course, a diminution of the tensions. Through the deposition of new material they soon, however, increase again in the dense stratum surrounding the kernel, and finally become sufficient to overcome the elasticity. For reasons already stated there then occurs, not a tearing of the layer into an inner and an outer part, but a straining, in consequence of which the starch substance in the middle of the layer becomes swollen and less light-refracting. The simple dense stratum becomes, in other words, differentiated into three; a median soft, and an inner and an outer dense.[1]

The peripheral dense stratum now behaves exactly like that which first arose through differentiation of the homogeneous grain. When the tensions have attained a certain intensity it experiences a strain in its middle, through which a soft stratum is produced—and so on.

Through the deposition of new material the inner parts of the starch grain, as a whole, become constantly more expanded by the outer. On the one hand there results from this a drag on the inner soft strata, in consequence of which they increase in bulk and in tendency to swell up. On the other hand it is also probable that dense strata are likewise affected, and the water in them increased.

The radial fissures, often present in fresh starch grains, as well as the partially compound grains, to be later discussed, are to be ascribed to the strain exerted by the outer parts upon the inner. That these clefts only depend upon non-uniform distribution of water is taught by the appearances which such grains present on slow drying. Those of beans, for example, which commonly exhibit gaping clefts, completely lose them on drying, the loss of water bringing about a diminution of the tensions. Since, however, the inner parts are richer in water and poorer in solids than the outer, they contract more than the latter on complete or nearly complete drying; they pass again, therefore, into their

[1] Compare Nägeli, *l.c.* S. 310.

earlier state of negative tension, and this is associated with the reappearance of the fissures.

More powerful swelling reagents, of course, bring about an increase of the tensions in each layer. *A priori* it is highly probable that the effort of the molecular layers to separate from one another would thereby be increased sufficiently to overcome the elasticity in fresh places. In other words, the original dense strata would experience in their middle a strain, and in consequence a stronger swelling of their substance; that is, would differentiate into three strata. This view again stands in full agreement with the fact that stronger swelling is associated with the occurrence of numerous new soft strata where none were previously visible; that is to say, where the tensions previously had not been strong enough to overcome the elasticity.

Strong swelling, however, is also associated with a considerable increase of the strain exerted by the outer strata upon the central parts of the grain; these, therefore, experience a stronger drag. We see in fact that the inner parts at first become greatly extended in a radial direction by means of the outer, and that at last they are forcibly torn from one another, so as only to present swollen fragments in a large central cavity.

In accordance with the foregoing, the differentiation of starch grains into regions of unlike wateriness presents itself as the necessary result of certain of their physical properties, and requires for its explanation no assumption of a growth by intussusception.

It need not be pointed out that cohesive or elastic properties, the action of mechanical influences upon the swelling power of starch grains, and finally the unequal extension in tangential or radial directions, are properties which the grain may acquire through growth by apposition as well as by intussusception; they alone are the grounds upon which my explanation rests.

That the capacity of the starch grain to lay down water in no way proves that it is permeable also for the dissolved substances out of which the grain is built up, needs no special discussion. We find, indeed, that the starch grain is not permeated by many solutions (for example of organic coloring matters), which are absorbed readily by cell membranes and protein crystalloids. Even assuming such permeability, we would be

far from justified in believing, on that ground alone, that there also occurred a change of the amylaceous substances into starch, and a deposition of the molecules so formed between those already present. We are just as little justified in assuming, before it has been definitely proved, that along with apposition-growth in starch grains some little growth by intussusception occurs, as we would be in making the same assumption with reference to a crystal of quartz or calc-spar.

In connection with the difference as to water contents between small starch grains and the inner set of layers of larger grains, the differences of form sometimes observed may be mentioned. Nägeli does not appear to have laid much weight upon these appearances, and only speaks of them very briefly.[1] According to him, in *Pisum* and other Papilionaceæ, the small grains are broader than the kernels of the full-grown grain. This is quite true if one compares the small and large grains of ripe seeds. The younger developmental stages of the larger grains have, however, no resemblance to the spherical or sub-spherical small grains which are present in ripe or nearly ripe seeds; they are thin, spindle-shaped, corroded, and resemble in form the nucleus of the large grains. In the root-stock of *Canna* are sometimes imbedded in the large grains "sets of layers of lancet-like or linear spindle form, such as no grains resemble in shape or structure." A figure is not given, but reference made to a similar structure depicted from *Cereus variabilis.* From this figure and from the description I believe myself able to conclude that we have here to do with an inner set of layers such as shown in one of my figures (Fig. 20); but we find in this case independent grains of the same form present, as Fig. 19 shows. I have not been able to examine the root-stock of *Dentaria.*

III.

According to Nägeli the unequal growth in different diameters of many starch grains is not compatible with external deposition. "It would be incomprehensible that free floating starch grains should increase seventy times more on one side than on the other."

[1] *Loc. cit.* S. 219.

The explanation which he gives of this phenomenon is somewhat indefinite. Its cause is to be sought in the arrangement of the smallest particles, and in the fact that on account of differences of cohesion in different places more material is deposited in some than in others.

The intussusception theory can give no very satisfactory explanation concerning the causes of this unlike arrangement and cohesion, which is a regular phenomenon in certain plants, and as regularly is absent in others; and for each species is so constant that in it only forms of one and the same type appear. With reference to it Nägeli's words are, "Since the nutrition depends not on external relations but on internal causes, the deviations which the starch grains show later in structure and form must be already present in their earliest beginning in the spherical smallest grains; this is conceivable, as the original spheres are formed under different specific relations. They show accordingly in the arrangement of their smallest particles, and in the nature of these, specific modifications from which of necessity the entire peculiar growth results."

The mode of growth of starch grains is, according to Nägeli, dependent only on internal causes; external influences could not bring about an uneven growth, but only exert a determining influence upon the direction of most or least growth. Excentric starch grains would grow most where they obtained the most dilute solution. This is, according to Nägeli, especially clearly the case in compound and partially compound starch grains, in which the directions of greatest growth are directed towards the centre of the grain. So also should be explained Crüger's[1] statement that excentric starch grains are attached by the hinder end to the primordial utricle or the protoplasm. "The close agreement of secondary grains and simple grains as regards increase of volume and density of the material along the long and short radii, supports throughout the view that the plasma in contact with the hinder end acts like starch substance, and, therefore, either entirely prevents the access of nutritive liquid or only allows a more dilute solution to pass." [2]

That the form of starch grains is primarily determined by the mode of nutrition, I have pointed out in a former work.[3] I have

[1] Bot. Ztg. 1854.　　[2] Nägeli, *l. c.* S. 327.　　[3] Bot. Ztg. 1880.

shown that centric starch grains arise when they are surrounded ring-like by starch-producing plasma (chlorophyl grain or "starch-former"); and that excentric grains arise at the periphery of the formative centre, and grow fastest at the points in contact with it.

The flat grains with central kernel originate in lens-shaped chlorophyl grains, and their broad sides are, as Nägeli has already pointed out, parallel to those of chlorophyl grains. The elongated starch grains of beans and some other Papilionaceæ are formed in spindle-shaped chlorophyl grains, with their long axis parallel to that of the latter. Flat excentric starch grains (e. g. *Canna*, *Phajus grandifolius*) are nourished by a formative mass ("starch-former" or chlorophyl grain) which courses along their hinder end. These phenomena can only be explained through unequal nutrition.

The relations between the growth of the starch grain and the supply of nourishing liquid are, finally, exactly what they should be in a body growing by apposition.

Excentric starch grains only touching the formative organ with one part of their surface, increase not only at this point; all or nearly all of the grain is recognizably in growth. This growth is fastest at the point of contact, and diminishes rapidly as the distance from this increases, so as to become extremely small at the anterior end of the grain, at least when the latter has attained a tolerable size. This point calls for more minute discussion.

If we seek to form a conception as to how a starch grain is nourished by its mother material, we can hardly conceive of the latter except in the form of a solution which impregnates the formative organ. We may leave it, however, undecided whether it is uniformly distributed through this organ, or (what is, perhaps, more probable in the case of peripherally originating starch grains) is limited to certain parts of this. In either case capillarity will lead to the accumulation of a layer of mother liquid between the starch granule and its supporter. The further necessary condition, that the nutrient matter shall not remain confined to this spot, is also fulfilled. A starch grain and its supporting formative organ, as we know, do not lie in the cell sap, but imbedded in protoplasm, and, as Hanstein first recognized,

the protoplasm is especially dense where in contact with the starch grain. If we imagine for a moment the starch grain and its nourisher surrounded, not by protoplasm, but by a jelly-like substance, then, through capillary action all around the starch grain, water would be drawn from the jelly and collect between the two in a thin stratum. This layer of water would necessarily be continuous with that collection of nutritive liquid separating the starch grain from its formative body, and would consequently obtain the properties of a nutrient liquid, and afford the starch grain with material for growth, diminishing in quantity with distance from the nutritive organ.

If, however, we assume that the water or watery solution impregnating the jelly is so combined with it as not to be capable of extraction by capillarity, then the layer of nutrient liquid between the starch grain and its formative body will, under the influence of the same force, spread all over the grain. In this case also the rate of growth would diminish with increase of distance from the formative focus.

Protoplasm, however, cannot, without much qualification, be compared to an ordinary jelly-like substance, and I, therefore, do not maintain that either of the above given explanations of the mode of nourishment of the starch grain is the correct one; though I think it highly probable. The illustrations mainly serve to show that so far as analogies are concerned we are led necessarily to a phenomenon of the same kind as that which we do actually find, and that for its explanation the assumption of an intussusception is by no means essential.

IV.

It is well known both partially compound and perfectly compound starch grains have yielded to Nägeli different points of support for the theory of intussusception. The following are those phenomena which, according to him, are not in harmony with the theory of growth by apposition.[1]

1. The difference of form between the secondary grains of a partially compound and perfectly compound starch grain on the one hand, and simple grains of the same size on the other.

[1] p. 223.

The former have hemispherical, angular, discoid, or elongated shapes, while the latter—the simple grains—are spherical. A development of these forms through fusion of simple isolated grains cannot be admitted, because the grains float free in liquid.

2. Whenever the secondary grains possess excentric kernels, these lie upon the outer side, away from the surfaces of contact of the secondary grains.

This position, aside from some peculiar exceptions which stand in precise relation with the irregular stratification of simple grains, is everywhere constant. The regularity would be inexplicable, however, upon the theory of apposition; at least the reason why the grains are always united by their posterior ends would not be apparent.

3. The occurrence of clefts between the secondary grains. The latter could not have been from the first enveloped in this manner by the external substance; the splits must have arisen subsequently—a point which, according to Nägeli, can be explained only through internal growth.

4. Specially important, according to Nägeli, are the differences in substance between the secondary grains of partially compound grains and simple ones of similar size. The latter are composed of comparatively anhydrous, the secondary grains, of watery substance.

The explanation which the theory of intussusception gives of these phenomena seems to me far from clear; at any rate, like Sachs,[1] I have been unable to grasp it.

" The conditions which disturb the concentric and radial arrangement of the component parts may reach such a degree in certain parts of the grain that the molecular forces of the surrounding stratified substance may be no longer able to control the new depositions. The latter then proceed in the same way as if they took place free in the cell fluid, where starch-forming goes on undisturbed by external influences. In this way is formed a complex of component parts which begins to stratify concentrically, and results in a secondary grain similar in its development to a perfect starch grain. These disturbing conditions find freest play where the molecular layers exhibit the greatest tendency to separate one from another, namely, close to the periphery in the neighborhood of sharp corners, edges and

[1] Exp. Physiologie, S. 421.

elevations, as well as in the centre of stratification itself, where, instead of one, two or more new kernels may arise."[1]

The formation of clefts between the secondary grains is to be regarded as a consequence of the weak cohesion at this spot, in which the arrangement of the molecules in the course of these secondary formations has suffered the greatest disturbance. The outer portions of the secondary grain are fed by a more concentrated solution than the inner portions; for this reason, the latter possess less cohesion and consequently exhibit more rapid growth.

The proofs that compound and partially compound grains originate by division and not by fusion of simple grains are in part no longer cogent. First of all, as regards the angular form of the secondary grains, it is indeed clear that it cannot be accounted for by compression of the grains. Nevertheless, the same phenomenon occurs in numerous organized bodies whose origin by apposition is undoubted; for example, to mention a case with which botanists are familiar, in the sphero-crystals of inuline, for which a correct explanation has been given by Sachs.[2] The flattening is due simply to this, that growth naturally stops at the surfaces of contact of two or more bodies which touch one another.

As to the greater softness of the contents of the secondary grains of partially compound forms in comparison with the contents of simple grains of equal size, this is the necessary consequence (precisely as for the contents of large simple grains) of the tension exerted upon the inner strata by the outer, and requires, after what has been said in the second section, no further remark or explanation. The splits between the secondary grains are doubtless to be ascribed to the same thing. That originally separate secondary grains which by subsequent growth come to touch one another and to be surrounded by common stratifications, adhere but feebly to their fastenings, and hence can easily be separated by mechanical conditions, is, *à priori*, probable, and is proven by this, viz. that compound grains which have undoubtedly arisen by the fusion of free simple grains break up easily under pressure into their secondary grains.

[1] p. 294, cf. also p. 323 *et seq.*   [2] Bot. Zeitung, 1864.

On the other hand it is far more difficult to reconcile with the theory of growth by apposition the statement that in the excentric secondary grains the kernels always lie on the periphery. If it were shown to be indeed true that forms such as those shown in Fig. 9 e have developed from forms like 9 a, the developmental history would furnish the most enigmatic contradictions. The rhizome of *Canna*,[1] where partially compound grains are very common, offers a superior field for investigations. In this plant the attempt was made to obtain a picture of the developmental history of the partially compound grains (Figs. 10–16). Close to the *punctum vegetationis* one finds in the first place only simple grains which, approximated in pairs or threes (at this level seldom more), are seated upon the "starch-formers." At somewhat lower levels, compound forms, having two or three members and usually a clear kernel, are abundant. That these have originated by the fusion of simple grains is put beyond doubt by their position upon the compound grains, by the corresponding disappearance of groups of simple grains made up of two or three members, and, finally, by the total absence of forms which might be looked upon as developmental stages between a simple and a compound grain. Stratification is present very early, but this is difficult to detect in the secondary grains on account of the marginal shadow. A little further from the apex of the rhizome, however, will be found some few stratifications (to some extent shared in common) upon the secondary grains. *The most vigorous growth will be found to have taken place, however, contrary to the statement of Nägeli, perpendicular to the axis joining the kernels and corresponding to the position of the "starch-former."* The comparison of the stages of development in sections taken further and further from the growing point demonstrates a steady subsequent development; the axis of strongest growth and the position of the kernels remain unchanged, at least in so far as the average distance of these latter can be made out in the partially compound grains of sections of varying age. In the full grown parts of the rhizome, finally, grains occur like that depicted in Fig. 16.

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[1] In Strasburg I used *C. gigantea*, in Baltimore a species unknown to me, but agreeing throughout with *C. gigantea* in respect to the starch grains.

The large grains having multiple kernels found in *Canna* and the potato, are, according to Nägeli, those which have just undergone division of their kernels; but one does not discover anywhere in the works of Nägeli upon what this statement is founded, and we are, therefore, justified in assuming that it is not a result of the comparison of starch grains found in sections of tissues of various ages, but that it rests upon purely theoretical assumptions, which, when once the theory of intussusception seemed to be proven by other phenomena, were justified, for then any other explanation was quite impossible.

Partially compound forms are indeed found in the rhizome of *Canna* having kernels far apart (Fig. 17 *b*), or again some in which the axes of strongest growth of the secondary grains are turned toward each other (Fig. 18.); these, however, are scarce in comparison with those having approximate kernels, and are easily accounted for by the fusion of two grains which either lay upon a single "starch-former," but at some distance from each other, or were produced from different "starch-formers." The starch grain depicted in Fig. 17 *b* is, for example, to be regarded as a more advanced developmental stage of a twin grain like that in Fig. 17 *a*.

The partially compound grains of the rhizome of *Canna* arise, therefore, from the fusion of originally free simple grains. The same explanation suffices for the grains of the pith-parenchyma of *Cereus speciosissimus*, which afford the most beautiful illustration of the same mode of development, since they actually exhibit the two angular corroded original masses imbedded in denser and not-corroded substance (Fig. 6 *b*).

Taking into account the phenomena above described, it seems almost certain that the partially compound grains, which are much more abundant in the potato than in *Canna*, and which have kernels removed far apart, have originated by the fusion of simple grains. Unfortunately, it is not possible in the potato, as it is in the *Canna* rhizome, to get a complete developmental history of these grains by the comparison of sections made through regions of different ages, so that we must be contented with the endeavor to answer the question how a fusion of two or more grains by their posterior ends could conceivably occur. In most organs of plants which possess excentric starch grains, the

chlorophyl grains or the "starch-formers" frequently exhibit, as
I have pointed out in my earlier work, starch grains lying at
two or more points of their periphery. Wherever two starch
grains lie opposite to one another, their posterior ends will natur-
ally be turned toward each other. The formative area gradually
diminishes when the starch grains have surpassed a certain size;
after a certain time only a thin stratum exists between them, and
this finally wholly disappears. Both grains have now fused into
one compound grain whose kernels are remote from each other.
The separate stages of this process can be followed without diffi-
culty in the rhizome of *Iris florentina*. Developmental stages
like those represented in Fig. 8 prove that the compound grains
in the potato which have kernels remote from each other, have
arisen in this way; the figure is taken from the rind of a young
greened potato. At *a* the greened "starch-former" is seen
reduced to a thin disk between the two grains; at the periphery it
extends beyond the grains as a thick swollen ring. Between the
secondary grains of the grain depicted in 8 *b* there is found no
longer any trace of the starch-forming organ; except a swollen
remnant of it which remains like a girdle around the basal
parts of the grains. This outer part of the starch-forming
organ will continue to form starch; since both grains touch each
other the newly-formed strata will be common to both, in other
words the compound forms will have been converted into
partially compound forms (Fig. 8 *c*). So far as concerns grains
like those depicted in Figs. 9 *a—d*, they can have originated
only by the early fusion of two simple grains, which lay upon
the starch-forming organs in an approximate rather than remote
position, as, for example, we have proven to be the case in the
grains of *Canna*.

So far as my observation goes, in *Phajus grandifolius* occur only
partially compound grains which have the direction of strongest
growth perpendicular to the line of union of the kernels. This
depends on the fact that in this plant the rod-shaped starch
forming organs bear starch grains only on one side; rarely, also,
upon their ends. A starch-forming organ may develop as many
as six starch grains, and these always lie in a line parallel to its
longer axis, never in the opposite direction.[1] The reason is the

[1] Cf. my paper in Bot. Zeitung, 1880, Figs. 36, 37, 39.

same for the rare occurrence in *Canna* of partially compound grains having remote kernels. Here also there is a localization of the starch formation upon one side of the starch-forming organ, so that I have observed only very rarely young secondary grains in an accidentally opposite position.

We have thus subjected to a closer examination all the phenomena advanced by Nägeli as points of support for his theory, and have seen that without the assumption of growth by intussusception they may all be explained in a simpler manner; while, on the other hand, there is a series of facts quite inconsistent with the theory of intussusception. We are, therefore, no longer able to ascribe to starch grains a molecular structure similar to that of protoplasm. Consequently our problem is next to determine to what category of bodies do starch grains belong.

V.

Starch grains possess no single peculiarity which justifies us in assuming for them a physical constitution very different from that of other rigid bodies; there are both among amorphous and crystalline bodies numerous examples of that characteristic peculiarity of starch grains, the power of swelling in water. The investigations of Schmiedeberg [1] and of Drechsel [2] as well as my own investigations [3] have shown that the protein crystalloids, which have so much resemblance to starch grains, can be produced artificially and represent the crystals of albuminoid substances. We have therefore merely to endeavor to decide whether starch grains are amorphous or crystalline bodies.

Those peculiarities which allow us best to distinguish crystalline from amorphous bodies, when definite crystalline form is absent, are cohesion and the optical properties. Hence in starch grains the solution of this question may be expected through the investigation of these peculiarities.

The peculiarities of cohesion (with which we may begin) have been already described in the second section; it has been shown there that starch grains are very brittle parallel to the strati-

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[1] Zeitschrift für phys. Chem. Bd. I.

[2] Journal für praktische Chemie, Bd. 19.

[3] Untersuchungen über die Protein Krystalloide der Pflanzen. Inaug. Diss.

fication, and vertical to it are very ductile. The difference is
so great that while radial fissures easily arise under the influence
of pressure, tangential splitting even by a destructive pressure
never occurs. A difference of cohesion in different direc-
tions has never been observed in amorphous bodies and is quite
inconceivable in them, since their chief characteristic is the
irregular arrangement of their parts. The splittings which arise
by crushing amorphous spherical bodies (for example dried gum
or caramel drops) take place very irregularly. The crushing
or bruising of fibrous crystalline bodies occasions, on the other
hand, the formation in the first place of fissures parallel to the
fibres, which means that the forces binding them together are
more easily overcome than the cohesion within the individual
crystals; the easy separability of the latter from each other
produces the striated structure which the surfaces of fibrous
crystalline bodies present, and which are also exhibited in a
striking manner by fragments of starch grains. Hence, starch
grains behave in respect to cohesion precisely like radially
fibrous crystalline aggregates (sphero-crystals), and differ entirely
from amorphous bodies.

The optical peculiarities are in full agreement with those of
cohesion; they are to be referred to the crystalline composition
of the starch grains, and not, as has been frequently assumed, to
tensions. These peculiarities have been the subject of several
erroneous statements, and on account of their importance for our
purpose must be more fully described here in respect to certain
details.

Nägeli has already sought to show that the cause of the double
refraction of starch grains is not the tensions; he believed that
he was justified in drawing the conclusion that double refraction
is not brought about by the tensions of stratification, because sec-
tions of the grain polarized light in the same way as when they
were a part of the intact grain.

This conclusion is, however, not justified, since doubly refrac-
tive bodies, which owe their polarizing peculiarities without ques-
tion to tensions of the same kind as we have in starch grains,
preserve these properties even when they have been broken into
little pieces (for example alum and analcim).[1]

¹ Marbach. Pogg. Annalen, Bd. 94.

That alum owes its doubly refractive peculiarities to tensions has been shown by Reusch,[1] who found that he could increase the double refraction as he liked, could diminish or could make it entirely disappear by an increase or diminution in pressure or traction.

Hence in alum the phenomenon depends on this, that the strata during solidification undergo a contraction in consequence of which the optical elasticity parallel to the surfaces of the crystal becomes less than it is perpendicular to them. A suspension of the tension, brought about by pressure, is accompanied by the disappearance of the double refraction, while traction in the direction of the surfaces brings about, on the other hand, an increase of tensions and hence also of the double refraction.

I have carried out similar investigations on starch grains; traction of the outer strata in the direction of the surface (since these, unlike alum, are in positive tension) must bring about a diminution of the tension, and in proportion as this takes place will the double refraction get weaker or wholly disappear, if it is dependent upon the tension. Starch grains which have been treated with very dilute potash undergo in the first place only a swelling of their inner softer substance, while the outer layers, remaining unattacked by the reagent, are nevertheless stretched by the swelling inner portion; the outer layers of grains treated in this manner did not thereby alter their optical properties, although the formation of numerous radial fissures must necessarily have brought about a marked decrease of positive tension.

Tensions therefore cannot be the cause of the doubly refractive properties of starch grains. Closer investigation, however, teaches, on the other hand, that the interference-figure in parallel polarized light in each individual case is exactly that which starch grains must exhibit if they were composed of fibrous crystalline (uniaxial or rhombic) elements whose course was similar to that of the splits, that is to say, perpendicular to the strata. Essentially, this conclusion has been put forward already by Baily.[2] On the other hand, the statement of Mohl,[3] that the

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[1] Monatsberichte der Berliner Akad. 1867; und Pogg. Annalen, Bd. 132. Groth, Physicalische Krystallographie, S. 117.

[2] Philosophical Magazine, 1876. Compare also V. v. Lang, Pogg. Annalen, Bd. 123 (and Carl's Repertorium, Bd. III.)

[3] Bot. Ztg. 1858.

arms of the cross of interference always run perpendicular to stratification, applies only to regularly symmetrical spherical grains; in excentric grains these often cut the strata at a very acute angle. In order in each individual case to determine before hand the *interference-figure*, one needs only to draw from the kernel to the periphery, lines perpendicular to the stratification. The dark bars will contain the parts of these lines which are parallel (or perpendicular as the case may be) to the direction of vibration of the Nicol.[1]

In regularly centred spherical grains, just as in the axis of excentric ones, the doubly refracting elements are straight and extinguish the light simultaneously along their whole length; on the other hand the case is different in the lateral parts of the excentric grains, where the fibres, as is shown by the splitting, take a bent course, and hence for every position, throughout a greater or less part of their length, dependent on the curvature, fulfil the conditions for the extinction of the polarized ray.

These peculiarities can be explained like those of cohesion, only upon the assumption that the starch grains are composed of crystalline fibres running perpendicular to the strata:

Starch grains differ from common sphero-crystals in respect to their power of swelling, hence we must call the fibrous crystals composing them *crystalloids*, as it is desirable to unite under this head all crystalline bodies which have the power of swelling.

As a result of these investigations, it turns out that starch grains are composed of radially arranged crystalloids, and exhibit the crystallization of starch substance, $C_6H_{10}O_5$, of which there are probably several isomers.

That the starch crystalloids always occur in the form of fibrous aggregates, and never single, can be referred to various circumstances. Previous investigations upon sphero-crystals have shown that the conditions for their appearance instead of separate crystals are, difficult solubility, feeble power of crystallization, and viscosity of the solution; to which, however, it should be added that a single one of these conditions is, in many

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[1] Still more simply by constructing a striation parallel to those parts of the layers whose course agrees with one of the directions of vibration of the Nicol's prism; this gives for each case a precise picture of the interference-figure, cf. Bailey, *l. c.*

cases, sufficient.[1]   We must leave it to be determined to which of these circumstances the regular occurrence of starch in sphero-crystals is to be ascribed ; we can, however, with some probability, assume that all three conditions are fulfilled.

That the strata in the tangential directions deposit more water can, I believe, in lack of a better explanation, be explained as conformable to the familiar hypothesis of Nägeli[2] concerning the form of the molecule, which he supposes to be longer in the radial direction than perpendicular to it.   That the strata are always formed perpendicular to the long axis of the fibres, as the course of the fissures shows, can also be simply explained by taking account of the fact that stretching is always easiest parallel to these fibres ; that in crystals the hardness varies with the direction, and has its maximum and minimum parallel to the crystallographic constants, may be assumed as already known.

Baltimore, *January*, 1881.

EXPLANATION OF THE FIGURES.

All the figures drawn with a magnifying power of 850 diameters.

FIGURE 1–3.—Starch grains from the cotyledons of the seeds of *Dolichos lablab.*

FIGURE 1.—Corroded starch grains from young seeds.

FIGURE 2.—Beginning of final starch formation around the corroded grains.

FIGURE 3.—Almost fully grown starch grains.

FIGURE 4–7.—Starch grains from the pith parenchyma of *Cereus speciosissimus.*

FIGURE 4.—Corroded grains from young cells.

FIGURE 5.—Commencement of final starch formation around the corroded grains.

FIGURE 6.—Grains surrounded by a continuous dense layer.

FIGURE 7.—Fully formed grains.

FIGURE 8–9.—Starch grains from potato.

FIGURE 8.—Chlorophyl grains with starch grains from the rind of a greened potato.

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[1] O. Lehmann, Ueber das Wachsthum der Krystalle (Zeitschrift für Krystallographie, Bd. I.)

[2] *Loc. cit.* p. 355.

FIGURE 9.—Partially compound grains from the interior of the same.

FIGURE 10–20.—Starch grains from the rhizome of *Canna gigantea.*

FIGURE 10.—Young starch grains on " starch-formers."

FIGURE 11–17.—Developmental stages of partially compound grains.

FIGURE 18.—Partially compound grain, with separated kernels.

FIGURE 19.—Narrow starch grain.

FIGURE 20.—A similar grain surrounded by strata of different direction.

FIGURE 21–22.—Starch grains from rhizome of *Canna sp.*

FIGURE 21.—Fresh.

FIGURE 22.—After swelling in dilute potash.

SOME OBSERVATIONS UPON THE FORM OF THE PULSE WAVE, AND THE MEAN ARTERIAL PRESSURE, IN A DOG WITH PATENT DUCTUS ARTERIOSUS.

By WILLIAM H. HOWELL, A. B., and F. DONALDSON, Jr., A. B.

In the course of some experiments which we were making upon the isolated mammalian heart, a dog evidently suffering from some form of heart disease came under our notice. We supposed that either the mitral or aortic valves were diseased, and Prof. Martin suggested that it would be of some interest to take tracings of the arterial pressure and the form of the pulse wave. It was especially desirable to know the arterial pressure, since such an observation, of course, could not be made upon the human subject except in a very indirect way.

A post-mortem examination which Dr. McLane Tiffany was kind enough to make for us, revealed the fact that there was a patent ductus arteriosus, establishing a very wide communication between the aorta and the pulmonary artery. There was also apparently some slight insufficiency of the mitral valves and of the pulmonary semi-lunar valves.

The aorta from its origin to the end of its arch was considerably dilated, though there was no evidence of any atheromatous changes in the walls of the artery.

The heart weighed 97 grams, and, upon comparison with the hearts of other dogs of about the same weight (from 15 to 18 pounds), showed general enlargement, together with some hypertrophy of the walls of the left ventricle. The heart of a dog of about the same weight from which tracings were taken for comparison, weighed 66.5 grams.

At the opening of the ductus arteriosus into the aorta there was a small valvular fold, not nearly large enough to cover the opening, but so placed as to direct the stream of arterialized blood sent out from the left ventricle at each systole along the aorta, and impede its passage into the pulmonary circulation; in form and

mode of action this valve somewhat resembled the eustachian valve of the fœtal heart. After the completion of the systole, however, when the elastic recoil of the aorta had set in, this valve could have offered no obstacle to the passage of blood from the aorta into the pulmonary artery; indeed, would rather have guided any backward current in that direction.

The only recorded case of this form of heart disease that we have been able to find, is the one reported by Dr. Hilton Fagge in the Guy's Hospital Reports, 1873.

As we were not competent to make a satisfactory auscultation of the case, we requested Dr. Frank Donaldson to examine the dog for us. This he very kindly consented to do, and gave us the following written report of the symptoms observed:

"I carefully auscultated the dog and found the heart beating at about 140 per minute; the impulse as compared with that of a healthy dog was much increased; the apex of the heart extended much further to the left of the sternum, showing marked hypertrophy. Over the whole cardiac region there was a loud, rasping, systolic murmur, with the maximum of intensity over the base; there was also a slight murmur with the second sound."

In our observations we endeavored in the first place to obtain tracings of the form of the pulse wave. The dog was tied down firmly upon a dog-board, and sphygmographic tracings were taken from the femoral artery by means of a Marey's sphygmograph.

The most favorable tracings obtained, when the animal lay perfectly quiet, and any irregularities resulting from psychic influences were excluded, were found, upon comparison with sphygmograms taken from the same artery in a healthy dog, to be entirely normal.

The femoral arteries were then laid bare and a cannula introduced into each of them; one of the cannulas was connected in the usual way with a mercury manometer, which served to register arterial pressure; the other was connected with a Fick's *federkymographion*. The object in using this latter instrument was to obtain some idea of the form of the pulse wave in the opened artery.

The pens of these manometers wrote upon the roll of paper of a Ludwig's kymograph and on the same vertical line; a chronograph

pen marking seconds and a Marey's tambour for registering res-
piration were also made to write upon the same roll of paper.

The animal was not at first under the influence of any anæs-
thetic, the operation of laying bare the femorals being too slight
to cause any serious pain ; afterwards chloroform was given. It
was noticed that when the animal was deeply under chloroform
the heart beats lost entirely an arhythmic character which had
been very marked when the dog first came under observation,
indicating that this irregularity had been caused before by
psychic influences.

The arterial pressure as given by the mercury manometer was
good, ranging from 140 mm. to 150 mm., which is within the
limits of what can be called the normal blood pressure of a dog.

The pulse wave given by the Fick manometer showed a sud-
den rise of pressure at the beginning of the wave, corresponding
to the sudden ejection of the contents of the left ventricle into
the aorta at each systole, and then a much more gradual fall of
pressure as the excess of blood in the arterial system was gradu-
ally forced through the capillaries into the veins, corresponding
to the description given by Fick of the pulse wave as obtained
by his manometer from normal animals. The descending limb
of the wave was marked by a strong indentation. This indenta-
tion or dicrotism is, according to Fick, who has made a careful
study of the tracings obtained from dogs by means of his mano-
meter, a characteristic of every true tracing, sphygmographic or
manometric, of the pulse wave. Roy, on the other hand, from
some experiments made upon rabbits with his sphygmo-tonometer,
says that the pulse wave in the opened artery is not, in a healthy
animal, dicrotic.

From a comparison of the tracings obtained from this dog
with others obtained from normal dogs, it was seen that the in-
dentation was more strongly marked in this case. In all other
respects the tracing was normal.

The pulse rate varied from 156 to 180 per minute.

The results of our observations, though mainly of a negative
character, are not on that account devoid of interest. The fact
that the animal kept up such an excellent arterial pressure is
especially worthy of notice. The normal pressure in the pul-
monary arteries of a dog, as observed by Beutner, Chaveau and

others, is not more than one-third as great as the pressure in the carotids. Knowing this, and remembering that the lung vessels possess great distensibility—can accommodate, in other words, a much larger quantity of blood than they usually contain without any rise of pressure in the pulmonary arteries resulting—and, further, that they are probably subject to vaso-motor influences to a much smaller extent than are blood-vessels in other parts of the body, one would conclude, from *à priori* reasoning, that when this very extensive and distensible vascular region was thrown into free communication with the systemic circulation, there would be a marked and permanent lowering of general blood pressure. That this did not occur must be explained by a compensatory increase in the force of the heart beat, or by an increase in the amount of peripheral resistance; or possibly by an increase in the total bulk of blood in the body.

The pressure in the pulmonary arteries must have been from two to three times greater than the normal pressure, requiring an increase in the force of the systole of the right ventricle to overcome this extra resistance, and causing a greater amount of blood to flow through the lungs in a given time into the left side of the heart. From the nature of the conditions governing the flow of the blood current in the aorta and in the pulmonary artery, it is not probable that there was any serious escape of venous blood into the systemic circulation; the abnormal flow must have been in the other direction—from the aorta into the pulmonary tract.

ON VARIATIONS OF REFLEX-EXCITABILITY IN THE FROG, INDUCED BY CHANGES OF TEMPERATURE. By W. T. SEDGWICK, Ph. D.

Physiologists are by no means agreed as to the effects upon reflex actions of changes in temperature. It is generally admitted that a cool temperature is favorable either for preserving or working upon reflex preparations, and that a warm temperature is equally unfavorable; but beyond and between these general and indefinite ideas there is a wide difference of opinion both as to facts and causes. This is the more surprising because looked at *à priori* nothing should be simpler. The organs combined to make up a reflex apparatus though now, in the adult, physiologically and structurally unlike, have all directly descended from similar protoplasmic masses in the embryo. Their tissues are composed, even in their highly differentiated conditions, of protoplasm more or less modified, and they should, therefore, obey less or more closely those laws which govern protoplasmic activity.

Every one knows that protoplasm wherever found behaves very definitely in respect to temperature. From almost complete inactivity at a low temperature it passes, with a gradual rise of temperature, little by little into a phase of greatest activity, beyond which under excessive heat its functions fall rather quickly back to zero, or if the temperature be raised still higher, pass beyond and disappear with the occurrence of coagulation and death.

It is agreed that most of the tissues and organs of the frog, taken separately, do obey the laws which govern their protoplasmic basis. Muscles, afferent and efferent nerves, and glands exhibit nearly the same series of events which may be observed in an amoeba or in a white blood-corpuscle. Even the heart—by no means a simple protoplasmic organ—is subject to the same laws when free from nervous disturbances. One fact of extreme

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importance must not be overlooked. Various protoplasmic combinations exhibit their periods of greatest activity at very different degrees of temperature. In some cases it might be supposed, therefore, that one portion of an apparatus would, perhaps, pass beyond its own period of activity before some other part would have reached the temperature best suited to it, thus causing the apparatus as a whole to behave in a contradictory or exceptional manner. It must be granted, however, upon the theory of the correlation of parts that it would be ordinarily more advantageous to the organism to have come to possess organs made up of harmonious than of discordant tissues; so that, unless evidence to the contrary is brought forward, we may reasonably expect to find in the parts of any apparatus no such dissimilarity in respect to their behavior toward changes of temperature.

It is within the experience of every physiologist that the frog, which, even in the normal state, is now admitted to be to a great extent a reflex mechanism, exhibits a noteworthy increase of functional activity as the temperature of winter gives way before that of summer. That the energetic movements witnessed in the summer, in the animal keenly alive to external stimuli, pass over in the autumn into the drowsy repose of the winter "sleep," is also known to every one. It is, therefore, somewhat surprising as well as confusing to read that in the brainless frog (a much more perfect reflex apparatus than the normal one) the motor and sensory nerves, according to most authors, obey the laws of protoplasm, while others state that the spinal cord exactly reverses them; to find that gentle heating of a reflex frog, in the opinion of one writer heightens the reflex excitability, and lowers it according to another; that packing of the body in ice increases enormously the reflex-excitability, and the same thing done with hot sand gives the same result; that the spasms of strychnia poisoning, commonly supposed to indicate a high-grade excitability, and which have disappeared in a room at the ordinary temperature, may be developed again in full force by laying the frog upon ice; while we are told that in spite of the fact that thermal stimuli are powerful agents for exciting reflex movements, a brainless frog will sit motionless until boiled, in water whose temperature is gradually raised. A

brief review of some of the literature of the subject will show that these apparent contradictions actually exist.

I. *Historical.*

Brown-Sequard[1] seems to have been one of the first to consider the effects of temperature upon reflex frogs. Having once succeeded in June in keeping such an animal alive much longer than usual, he was led to observe again in September and later, and found at length that he could keep frogs, etc., in good condition during these months for days and even weeks after the destruction of the medulla, while previously an hour or two was the longest time observed. He also noted the effects of destruction of portions of the cord; and when the objection was raised that very likely the prolonged vitality detected by him in the autumn was due only to the same actual amount of energy fading out more slowly (owing to the retardation of functional activity by the lower temperature), he replied by advancing experimental evidence that there is really more energy exhibited in the fall than in the summer—a more prolonged and vigorous vitality rather than a longer exhibition of an enfeebled vitality.

Kunde,[2] writing a revised account of his previous work, states that if a frog be cooled, an electric current which, when the frog was warmer, produced tetanic movements, now either produces them later or not at all. He investigates the effects of temperature upon the spinal cord by giving frogs strychnia and then placing the animals in water at different temperatures. From his researches he concludes that frogs under small doses of strychnia, lose their spasms in the cold and regain them when brought back into a warm room. A dose just large enough to produce spasms in a warm room having been given, the animal was put upon ice and the spasms disappeared. If the animal were held in the hand of the observer or carried back into the warm room they returned. Large doses have precisely the opposite effect. The spasms in this case having disappeared under heat, will reappear in the cold. His work, then, indicates that cold depresses reflex excitability, except in severe strychnia poisoning.

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[1] For titles see list of references at the end of this paper.

Cayrade,[3] writing in 1864, states that heat shortens the duration of reflex movements, but increases their energy. When the increase of temperature is gradual, "as in nature," the reflex functions also increase gradually their functional activity ; "movements are more speedy, more energetic, and contractions last longer." When the temperature is "very high, 29°–30°, for example," section of the medulla produces tetanus and convulsions ; from which it appears that his conclusions given above are drawn, in part at least, from intact frogs.

He believes that a sudden rise of temperature is depressing in its effects upon the reflexes, an opinion derived from his consideration of Kunde's earlier work (1857), in which a frog poisoned to tetanus at the ordinary temperature, lost the spasms and recovered at 34° : also from this observation ; if two cats of equal weight be poisoned with the same-sized doses of strychnia, and if, when tetanus has appeared, one be left in a room at the ordinary temperature (16°–19° C.) while the other is put in a room at 40° C., the former speedily dies, while the latter gradually recovers. He closes the subject with the remark that in order to work upon frogs in the summer, one must keep them covered with wet linen, which keeps them both cool and moist.

Weir-Mitchell[4] and *Richardson*[5] published in 1867 communications on the effects of extreme cold (freezing by ether and rhigolene spray) upon frogs and some other animals. Incidentally they remark that the freezing, if not too sudden, was the cause of a preliminary stage of increased excitability, though this speedily passed into total loss of function, if the whole animal were frozen, or if all of the cerebro-spinal axis were affected. They observed that frogs and rabbits having frozen brains behaved in respect to their reflex actions precisely as if they had been decapitated, *i. e.* the reflex-excitability rose enormously.

For the purpose of demonstrating a striking difference between the normal and the brainless frog in respect to conscious sensation, *Goltz*[6] in 1869 recalled an experiment described by him long before that time. Though employed by Goltz for a quite different purpose, it is nevertheless of great interest to us, since it has given rise to no small difference of opinion concerning the effects of heat upon reflex excitability. Goltz's experiment is as follows: A normal frog if immersed in water which is

gradually heated, speedily becomes violent in his attempts to escape. In striking contrast to this phenomenon is the behavior of the brainless frog, which, on the contrary, save for a few unimportant twitches, sits motionless until it is dead from the excessive heat. Though Goltz makes no definite statements as to the cause of this singular quiet of a highly excitable reflex frog (a matter which has been studied by Foster *et al.*), it seems fair to conclude from the context that he refers it to a dullness of perception which is not present in the frog possessing a cerebrum.

Tarchanow [1] studied in the first place the effects of heating and cooling sectional areas of the central nervous system. For this purpose he used either high or low temperatures (heated oil or ice) and thus applied powerful stimuli. His results indicate a marked coincidence between chemical or electrical and thermal stimuli. Besides, he devised the following important experiment :

" If the spinal cord of a decapitated frog be laid bare along its length and covered with ice or snow, a definite depression of the tactile reflexes will be noticed. If, on the other hand, the cooling take place upon the intact trunk of a frog similarly decapitated and without any opening of the neural canal, we obtain results diametrically opposed to the foregoing, *i. e.* a quite clearly pronounced increase of reflex excitability."

In order to effect this, he recommends that the trunk of the frog be packed in ice, by means of a bag or sack having holes below for the hind legs.

Tarchanow has also studied upon the normal frog the effects of heating and cooling, and employed for the purpose, apparently not knowing of Goltz's work, the same method which was devised much earlier by that observer. He notes the period of unrest through which the animal passes as the temperature rises, and also the period of prostration which finally ensues. He calls attention to the fact that since the cause of this prostration cannot lie in the nerves or muscles (these being found intact), it must be sought in the brain or spinal cord. By certain experiments not wholly free from objection, he concludes that the cause lies in the brain and not in the spinal cord. He points out again that direct cooling, by ice or snow, of the exposed cord, as described above, gives a depression of excitability. Indirect

cooling by ice-packing gives an enormous rise of that excitability, but he omits to explain this difference, as early in the paper he promises to do, and leaves it without further remark.

Heinzmann,[8] working under the guidance of Preyer, published in the next year (1872) a paper of very great interest to the student of this subject. Starting from the fact that a motor nerve may be subjected to stimulation (chemical, electrical, pressure, and heat and cold stimuli are mentioned) too feeble to excite movement of the connected muscle, and that this stimulation may be gradually increased in intensity so far as to produce finally destruction of the nerve and yet without causing the least movement in the muscle, Heinzmann raises the question as to whether or not the same thing is true of sensory (afferent) nerves.

Thermal stimuli seemed to offer the best opportunity for the examination of this question, and by means of a carefully arranged apparatus the work was begun.

Normal frogs and frogs destitute of cerebral hemispheres were heated very gradually both " locally " and " totally." The local heating was by dipping one leg of a frog hung by the jaw from a hook, in water whose temperature could be gradually raised or lowered. In the " total stimulation " the whole body was heated by allowing the frog to sit upon cork floating in a cylinder of water which could be heated gradually. A uniform result was obtained.

The frog destitute of cerebral hemispheres could be heated easily, the normal frog for obvious reasons with some difficulty, until death ensued; often passing from, perhaps, 22° C. to 40° or 45°; or could be cooled as many degrees with a similar absence of movement. This result seemed to Heinzmann satisfactory. It put the sensory alongside the motor nerve in this respect, and seemed only to add another support to a well-established law. Heinzmann's conclusions in regard to the " total " heating of the normal frog must be compared with those of Goltz and Tarchanow, who both found, unlike Heinzmann, that gradual heating of the normal frog produced most violent movements. Heinzmann does not refer to the work of either of these observers, and apparently does not know that in recording the quiet of the headless frog under a gradual rise of temperature he is but repeating a much earlier experiment of Goltz. It must not be overlooked

that his explanation of the phenomenon differs widely from that which might be inferred from Goltz's paper. The latter's work seems to imply that the quiet of the brainless frog is due to dullness of perception, so to speak, while Heinzmann sees in the phenomenon a failure to secure movement due merely to a lack of stimuli succeeding each other with sufficient rapidity.

Heinzmann has also undertaken to fix the nearest temperatures at which reflexes appear in frogs of known warmth under heating or cooling of fixed rapidity (*Reflexschwelle*), and the rapidity of stimulation needful to provoke movement at various temperatures (*Unterschiedsschwelle*).

In 1872 appeared in the Russian language a paper by *Tarchanow*[9] on the physiology of thermal reflexes. I have not seen the original, but have been obliged to depend for an abstract of it upon the *Jahresbericht* of Hofman and Schwalbe for 1872.

The author compared with each other the sensibility of the skin and afferent nerve, and concluded that special end-organs for the detection of thermal stimuli must be located in the skin. Setschenow had already advanced the idea of special end-organs for the detection of chemical stimuli, and others have located there tactile end-organs, so that Tarchanow remarks that it only remains for the microscope to detect the structural peculiarities of these three kinds of nervous end-organs. He has noticed the unrest of the frog destitute merely of the cerebral hemispheres, already observed by Goltz long before. Finally, having observed that warm dilute acid (in Türck's method) called forth reflexes sooner than the same acid when cool, he proceeds to draw from the fact two interesting conclusions: 1. " This result can be explained by the hypothesis that by the higher temperature the irritability of the nerve-endings in the skin is increased." 2. " In this way, probably, is to be explained the well-known fact that on passing from a warm into a cooler medium the animal reacts more quickly than when passing from a cool into a warmer medium ; in the former case the end-organs are in a more irritable condition."

Dr. M. Foster,[10] in 1873, raised the question why, in the experiment of Goltz described above, the brainless frog (a far better reflex machine than the normal one) remains undisturbed in water the temperature of which is gradually raised. Goltz

argues for a greater dullness of perception in the brainless frog, because it sits quiet under conditions which throw the normal frog into violent movements, viz. under a rising temperature; but he does not mention that we have a strange anomaly in the fact that the normal frog, whose reflex functions are feebler than those of the decapitated animal, reacts much sooner upon the application of heat-stimuli. I shall shortly return to this paper, so that at present it need only be said that Dr. Foster, who apparently had not seen the paper of Heinzmann, published a year before, came to a result wholly different from that author. Heinzmann believes the quiet of the reflex frog to be due to a lack of stimuli-changes succeeding each other with sufficient suddenness; Foster, on the contrary, believes the spinal cord to be directly depressed in function by the hot circulating blood.

In the same year (1873), appeared in the Russian language an article by *Archangelsky*, [11] on the influence of warmth upon the nervous and blood-vascular systems of the frog. Of this paper I have seen only an abstract given in the *Jahresbericht* of Hofman and Schwalbe for 1873. Archangelsky used in his work, as a convenient means of regulating the temperature, a wooden box having two windows, and provided inside with tubes arranged zigzag upon its walls, so that they presented a large surface to the air of the chamber and could be filled with hot or cold water at will. He seems to have worked first upon normal frogs; and he found that when these were warmed to 29°–34° C., cramps were readily observed, succeeded by weakness, inaction and heat-rigor. He remarks that it is a matter of indifference whether the air be moist or dry, the end-result being the same. When it is moist, however, a high temperature is much sooner reached.

Upon decapitated frogs he has investigated according to Türck's method the variations of reflex-excitability. He does not say whether the air was dry or moist in this case. Under rapid heating he finds the excitability, at first, heightened; under slow heating he was able to discover no change in the irritability at the outset. He finds "in many cases" that when the temperature has reached 25°–30° C. the reflexes evoked by acid become gradually weaker and finally cease, though the tactile reflexes remain somewhat longer (33°–34°). The acid and the water for removing it from the foot were kept inside of the warm chamber.

Archangelsky has also studied, in respect to its behavior toward warmth, the reflex apparatus analyzed into its separate parts; having sought in this way to locate the cause of the failure of reflex power under heat.

(*a*) *The end-organs.* "It proved to be the fact that the higher the temperature of the acidified water, the sooner were the feet withdrawn. Hence the excitability of the end-organs is heightened by heat."

(*b*) *The afferent nerve.* Like other observers, the author finds the afferent nerve to be more irritable when warmed; he says, however, that slow warming has no perceptible effect.

(*c*) *The spinal cord.* Two needles having been thrust into the cord of a decapitated frog, were connected with an induction apparatus. As a measure of the excitability, that distance of the secondary from the primary coil which was just sufficient to produce a minimal contraction of the muscles, was employed. The result proved to the author that the irritability gradually falls and becomes zero at 34°—the very point at which the reflexes, under similar conditions, also disappear. No preliminary phase of increased activity is mentioned; nor is it stated whether or not the air was saturated with water. As a check upon this experiment, more evidence was sought in this way: a brainless frog was hung up in a glass tube, which covered only the upper part of the trunk and left the pelvis and legs not covered in any way. Including the tube just mentioned and connected tightly with it was a larger glass tube of the same form. Thus a hollow jacket was formed around the frog and yet not touching him, and through this jacket could be passed water of different temperatures. It turned out that rapid heating produced at first a rise of excitability (measured by Türck's method) which speedily passed over into a fall even to zero; while gradual heating produced a steady fall, with no previous phase of heightened excitability.

(*d*) *The efferent nerve.* This was investigated with the results already reached by numerous observers. Like the afferent nerve it preserves its irritability at a temperature above that at which the reflexes fade away.

(*e*) *The connected muscles.* These were investigated with the well-known result. The author found, however, that in dry air

a muscle did not pass into rigor before 45°–50° had been reached; whilst in moist air it perished at 33°–35°. Its irritability in a moist room quickly increases from 20°–30° and then gradually decreases to 34°.

Archangelsky's conclusion is easily foreseen. The loss of reflex excitability under heating is due, according to him, to a weakening of the spinal cord alone.

Like Heinzmann, *Fratscher*,[12] working in 1875 in the Jena laboratory under Preyer, does not at the outset undertake to contribute to the discussion of the present problem. Heinzmann having reached the extremely interesting results described above, it was an important question to ask if acids and alkalies might also be so stealthily administered to a part of a living animal (either brainless or normal) as to cause destruction of tissue without having ever produced movement. This question Fratscher took up under the direction of Preyer, and he had already succeeded, as he believed, in demonstrating the truth of the hypothesis, when, by Dr. Foster's paper, his attention was called to the explanation of the effects of thermal stimuli gradually applied, and to the need for a repetition of Heinzmann's work. This he undertook, and he reiterates all of Heinzmann's results, contradicting some of Foster's statements in a way which will shortly be described. He finds that heat stimuli, as well as acid and alkali stimuli, if only applied slowly enough, may be concentrated so far as to produce tissue-death without giving even a solitary movement.

Rosenthal,[13] in a brief summary of his " Studies on Reflexes," published in 1875, states as one result of his work, that cooling depresses reflex-excitability. This result, it will be observed, is practically opposed to the conclusions of Tarchanow, Foster, etc.

In the same year, *Freusberg*[14] makes use of the experiment of Tarchanow[7] quoted above, and verifies it. He endeavors to explain it upon his theory of " latent stimulation," and, what is of great interest, shows that not only will ice-packing enormously raise the reflex-excitability, but that packing in hot sand will do the same thing. (cf. Archangelsky.[11])

He distinctly affirms that an explanation is not to be sought for in a general reduction of body temperature, " for this, on the contrary, effects a general inactivity of the organism; and

besides, the phenomenon is so quickly produced by the ice-packing that it cannot be ascribed to that cause."

Freusburg was at once attacked by *Tarchanow*,[15][16] who refused to accept his explanation of the increased excitability seen in a frog packed in ice. Tarchanow states that he prefers an explanation offered (he omits to say where) "already some years ago," and states, as showing the falsity of Freusberg's theory, that an exsanguinated frog does not show the same phenomenon which he observed in 1871. From this observation he concludes that the blood is an essential element in the experiment, and seeks to account for the facts by supposing that the heightened excitability is due to an excess of oxygen, the result of cessation of rapid oxidations, or by considering a deficiency of CO_2 as the active cause, etc., etc.

Freusberg[17] returns the attack by showing defects in Tarchanow's method and obscurity in his results. He seems to me to have decidedly the best side of the question.

The second part of *Wundt's*[18] *Untersuchungen* appeared in 1876, and contains one section devoted to the influence of temperature and the time of year upon the reflex-excitability of the frog. He has worked, however, only upon the effects of lowering, and not upon the effects of raising the temperature. By employing methods similar to those of Tarchanow[7] and Freusberg[14] (mentioned above) he has substantiated and somewhat extended their results. By ice-packing of the trunk he obtains, like them, an increased reflex-excitability, which, however, passes over speedily into gradual depression, and finally into a condition of complete inactivity under stimulation.

His explanation of the phenomenon is somewhat unlike Freusberg's, which apparently he had not seen, and cannot be given in full at this point. He considers it, however, as due partly to heightened activity of the motor nerves and partly to central nervous changes. He further points out a singular effect of cold upon the spasms caused by strychnia. He affirms that, as is well known, a small dose will produce violent spasms at the ordinary temperature, while he adds that even large doses produce no effect upon a frog in the cold. It is interesting to compare these results with those of Kunde[2] given above.

In closing, Wundt suggests that his experiments seem to offer a sufficient explanation of the various changes which frogs undergo in respect to their reflex-excitability during the various seasons.

The latest contribution to this subject, so far as I know, is embodied in a suggestion offered by *Langendorff*.[19] This writer found that stimuli appear normally to travel along the optic nerve, and to inhibit reflex actions to some extent, perhaps by exciting the so-called "inhibitory centres" of Setschenow. He recalls an observation of Fubini, that after blinding the reflex-excitability of a frog is increased, sees in it a confirmation of his own idea, and adds that he is inclined to believe the rise of irritability after ice-packing, observed by Tarchanow[7] and Freusberg,[14] to be due to an anæsthesia of the skin, which, if I understand him, no longer sending in exciting stimuli to the inhibitory centres, allows them to relapse into quiet, and thus brings about heightened excitability.

The writer of the present paper was led to take up this subject by a perusal of Dr. Foster's article referred to above, and more especially by certain evidence and conclusions recorded by Dr. Foster which seemed to be scarcely harmonious with well established physiological laws. The results of his investigations (which have now extended over a considerable period) have justified him, he believes, in making still further studies. The present communication will be devoted chiefly to a review of certain parts of Dr. Foster's paper, and to the description of some new observations bearing upon the problems at stake.

II. *The Experiment of Goltz.*

As has been stated above, it was pointed out long ago by Goltz that the brainless frog, if allowed to rest in water the temperature of which is gradually raised, behaves wholly unlike the normal frog under the same circumstances. The normal frog leaps away, or, if confined, becomes violent in his attempts to escape as soon as the temperature of the water reaches 30° or thereabouts, while in the same vessel the brainless frog sits motionless until death supervenes.

This observation was repeated and verified by Dr. Foster,[10] who saw, however, in the behavior of the brainless frog a new problem which had not been touched by Goltz. Goltz's experiment no doubt demonstrates as clearly as he meant to have it, a difference between the normal and the brainless frog; but, as Dr. Foster observes, it presents a new difficulty, viz., " why the brainless frog is not excited to reflex action by the stimulus of the hot water?"

It might have been expected that a frog in full possession of his faculties would be more acute than a brainless frog in perceiving a temperature which was gradually rising to a painful pitch, and more prompt and skilful in his endeavors to escape than his neighbor destitute of a brain and scarcely recovered from a recent profound operation ; but it would not have been predicted that a decapitated frog, whose reflex functions are well known to be keenly alive and even more delicately adjusted and more easily aroused than those of the normal frog, would sit unmoved in the presence of abundant stimuli until it perished from excessive heat.

It is a surprising fact that although provided with a delicate reflex apparatus, ordinarily responding to small heat stimuli quite as well as to acids or mechanical injury, the brainless frog remains perfectly calm in the presence of multitudes of powerful stimuli which are attacking large areas of his sensitive skin, and makes not a single reflex movement worthy the name. Still more astonishing is it when we learn that during this period of calm, very complex and orderly reflex movements can be evoked by a gentle touch or a drop of dilute acid, proving that the reflex apparatus is not paralyzed, but, for some reason, though wide awake to other and apparently feebler calls, is deaf to those of the heat stimuli.

This problem which Dr. Foster has pointed out he has also endeavored to solve. He has extended and modified Goltz's experiment, using for the purpose brainless frogs suspended by the jaw, and immersing the hinder parts to various depths in water whose temperature could be gradually raised. In this way various definite areas of the body-surface could be exposed to the action of gradually-heated water, and his results are described by him as follows:

"*Observation* 1. If a frog, from which the brain has been removed, be suspended by the jaw, with the legs hanging freely down and the toes dipping into a vessel of water, on gradually heating the water the toes are withdrawn by reflex action as soon as the temperature of the water reaches a little over 30°. The result does not essentially depend on the rapidity of the rise. However slowly the water be heated, the feet are always withdrawn at a temperature of 35° or earlier. Rapid heating may possibly lower the degree at which the feet are withdrawn ; but to this I have not paid particular attention. Whether heated slowly or rapidly, the feet are withdrawn at about 35° C. or at a lower temperature.

Observation 2. If the whole body, thus suspended, be similarly immersed and heated, no movements (or only the very slightest spasms of the muscles of the legs) take place ; and on still further raising the temperature, the body becomes rigid (*rigor caloris*).

Observation 3. If both legs be immersed up to the anus and similarly treated, they also become rigid without movement either of the legs or of any part of the body, save only a few spasms.

Observation 4. If one leg only be immersed and similarly treated, it also becomes rigid without movements, or with only slight movements.

Observation 5. If both legs (or one leg) be immersed up to the knee, they are sometimes withdrawn, but sometimes no movements take place, and the portion immersed becomes rigid. The results in this case are not so constant as when either more or less of the body is immersed.

Observation 6. If the feet only are immersed, they are invariably withdrawn at 35° or under.

Observation 7. If a frog be suspended over a vessel divided by a partition, with water at unequal levels on the two sides, so that one leg is wholly immersed and the foot only of the other leg, and the vessel be surrounded with water the temperature of which is gradually raised, neither the leg nor the foot will be withdrawn, if care be taken that the water on both sides of the partition be equally and uniformly raised in temperature. If, in this last observation, the water on both sides be reduced to the same level, both feet are withdrawn. This result shows that warm air and vapor have not the same effect as warm water, and that the absence of movements is not due to the unavoidable contact of the thighs of the animal with the top of the partition giving some support to the legs, and thus diminishing the tendency to the withdrawal of the feet."

It is not difficult to repeat these experiments and to arrive at about the same results. It is, indeed, my own experience that if no special attention be paid to the rate of heating, and that if it be not too rapid, one will obtain results agreeing essentially with Dr. Foster's. If, for example, a brainless frog be immersed as above described in water at 20° or 18° C., and the temperature be raised to 40° (by a lamp below the vessel) in ten, fifteen or twenty minutes, events will justify the above statements.

If, however, a powerful burner be used and the water be heated in much less time than ten minutes, not even the frog immersed to his fore limbs will remain quiet, but, like the frog with only his feet immersed, will exhibit violent movements. It is easy to prove, and is practically admitted by all observers, that under heating which is at all entitled to be called " gradual," the immersion of an actively reflex frog suspended as described above and immersed to the fore limbs or to the anus, will bring about such a state of things that the animal will pass into heat rigor without making a single movement of consequence.

A year before Foster's work was published, *Heinzmann* [8] had found that by gradual heating of an entire frog, or even of only one hind leg, the temperature of the animal or of the part might get to be so high as to produce rigor and yet without the least disturbance of its general repose. He, however, puts special stress upon the effects of very gradual heating, and makes the important discovery that even a normal frog may be made to perish in the same way without a struggle, provided only that the increase of heat be gradual enough. This statement involves a direct contradiction of the statements of Goltz,[6] Tarchanow [7] and Foster,[10] who have all agreed that under gradual heating the normal frog becomes violent in his attempts to escape. The contradiction is only partial, however, for any one in half an hour can prove to his satisfaction that the three observers are correct ; while *Fratscher* [12] has fully justified Heinzmann. The truth appears to be that if the heating be sufficiently gradual, no reflex movements will be produced even in the normal frog; if it be more rapid, yet take place at such a rate as to be fairly called " gradual," it will not secure the repose of the normal frog under any circumstances, though it will do so for the reflex frog if only enough of his skin be immersed, while

it will fail if only a small portion be dipped; again if the temperature rises so rapidly as scarcely to be called "gradual" in its upward progress, not even the reflex frog will remain quiet, though wholly immersed, but, like the normal frog, will exhibit violent movements.

Heinzmann did not experiment with immersion of the feet only, so that an interesting question was left after the paper of Dr. Foster appeared, as to whether or not Heinzmann would have succeeded in keeping the frog quiet by his extremely gradual heating had he immersed only so small a portion of the animal as the feet. This question has been answered in the affirmative by *Fratscher*,[12] who found that he could warm even the normal frog to the point of rigor by immersing merely the feet. My own work points in the same direction; and we may take it as settled that Foster was mistaken when he came to the conclusions laid down in *Obs.* 1. I believe that I can explain, however, the result which Dr. Foster obtained. In my own case, at least, I found that it was due to reflex movements, caused by drying. When the feet only are immersed a very large part of the body is exposed to the dry air of the room, and the naturally moist skin of the frog dries, producing reflex movements. In a moist chamber it is not very difficult to raise the temperature of the water in which the feet are dipping, higher than 35° without causing movement.

It is plain from what has been said that the smaller the portion of the animal immersed the more difficult it is to heat without producing movements, and the more gradual must be the rise of temperature. Moreover, since, as the part immersed gets smaller, the surface exposed to outside stimuli gets larger; while, at the same time, the heating must be more gradual (thus prolonging the period of exposure) and the tendency to movements gets greater, the slight stimulation due to drying, and perhaps to the coincident cooling of the not immersed parts, becomes an important factor in the experiment; a factor which is less important and can be neglected when much of the body is immersed, but which may lead to error when the feet only are dipped. At least one safe conclusion may be drawn at this point. It is plain that if Goltz had slightly varied the conditions of his experiment; if his brainless frog had not been in contact with

the heated water by a tolerably large surface, he would have failed to demonstrate by this experiment that difference between the normal and the headless frog for which he was seeking.

We have next to consider why it is that the reflex (and the normal) frog, in full possession of healthy end-organs to detect and sensory nerves to transmit painful impressions, may nevertheless exhibit total indifference to temperatures which are gradually raised so high as to kill the tissues immersed. Different explanations have been offered by Goltz, [6] Heinzmann [8] and Foster [10] respectively, and that of Goltz may be conveniently referred to first. I have not seen his original communication upon the subject, but if one may judge from the context in the description of the experiment given in 1869, it appears that Goltz [6] considers the lack of movement to be due to lack of " perception." He regards the failure to move under abundant stimuli as showing this lack of perception, not wanting in the normal frog, which therefore displays movements. If this be the theory of Goltz to account for the quiet of the reflex frog it is plainly defective, since the reflex functions of the brainless frog surpass in delicacy those of the normal one.

The theory of *Heinzmann*, [8] who approached the subject from an entirely different standpoint and while endeavoring to solve a different problem, may conveniently be deferred until the theory of *Foster*, [10] who was, I believe, the first to raise the point at issue and who has given the subject its most exhaustive treatment, shall have been reviewed.

After describing the results of his investigation in the passage quoted above, Dr. Foster writes as follows (p. 46):

"The above observations show that when the toes (alone immersed in water) begin to be affected by the high temperature, say 30° C., the stimulus of the hot water causes a reflex action which results in the withdrawal of the foot. When the whole leg or body is immersed, the same stimulus is still at work, but no reflex action occurs. What is the reason that reflex action is absent?

The following explanation is, perhaps, the first to offer itself. The warmth applied to the leg diminishes the irritability of the nerves or of the muscles, or of both; and thus the impulses generated by the warm water in the sensory terminations of the nerves of the foot are not carried up to the cord, owing to the diminished irritability of the

sciatic trunk, or, being so carried, the reflex process taking place in
the cord cannot manifest itself on account of the diminished irrita-
bility of the muscles or motor nerves.

But this view is clearly untenable. It requires that the nerves and
muscles, covered and protected by the skin, should be affected before
the sensory terminations in the skin itself. Moreover, no appreciable
difference in the irritability of the nerves, trunks or muscles of a leg
thus exposed to 35° C. could be detected. And it is directly contra-
dicted by *Obs. 7*, where the immersion of one leg prevents movements
in the other.

Two other views then suggest themselves.—(1) The blood return-
ing from the legs being warmer than the normal, raises the tempera-
ture of the spinal cord above the normal; this reduces the irritability
of the cord, and hence reflex actions set going by a feeble stimulus,
which in a normal cord would manifest themselves, are here absent.
(2) From the stimulation of the whole leg as compared with that of
the foot, a multitude of impulses, arising from all parts of the skin
exposed to the warm water, reach the spinal cord. These produce
such an effect upon the cord that the simpler reflex action resulting
from the stimulation of the toes alone is prevented."

It will be observed that the question raised in the first part of
the passage here quoted, in view of what has been said above,
would now have to be stated somewhat differently; nevertheless,
the question is at bottom much the same, viz. why the frog is
not excited to reflex action by the stimulus of the hot water. It
may be well also to recollect, at this point, that the rapidity with
which the temperature may be raised without causing reflex
movements seems to depend largely upon the amount of surface
immersed.

It will be instructive to follow the evidence which leads Dr.
Foster to accept as the principal cause of the phenomenon in
question, the former of the two views which he has suggested.

III. *Is it true that the brainless frog sits motionless in water
which is gradually heated, because the irritability of his spinal
cord is depressed by heat brought by the blood from a remote part
of the body?*

This is the theory finally adopted by Dr. Foster; hence it
must be specially examined. It involves one very conspicuous

objection, however, which Dr. Foster has not overlooked, but which he dwells upon in these words (p. 50):

" In all observations on the effect of a rise of temperature on living animal tissues, the state of exhaustion or depression which ultimately ensues is preceded by a stage of exaltation in which the functions of the tissue are raised above the normal. This is well shown in the case of muscles, nerves and the heart. In none of the observations recorded above was there any indication of such an initiative stage of increased action. Had there been it would naturally have led to the withdrawal of the feet in all cases. And the absence of this presented a great difficulty to considering the results obtained as being merely due to a depression of the powers of the spinal cord by reason of the increased temperature.

" Some observations, however, made in the laboratory here by Mr. T. O. Harding, afforded a clue, by pointing out a distinction between simply and directly raising the temperature of an organ or a tissue, and indirectly heating it by supplying it with blood heated beyond the normal in some distant part of the economy. Thus the heart of a frog, either empty or filled with serum, when heated beats with a more frequent rhythm and, at first, with greater force. But the same heart when indirectly heated by the immersion of the legs of the frog in hot water (the heart remaining in the body and the brain and spinal cord being destroyed) is lowered at once both in the force and frequency of its beat, by reason of the heated blood with which it is supplied. This result leads us to expect that in the same way the spinal cord, if heated by being supplied with blood heated beyond the normal, would be depressed without any preceding stage of exaltation, and thus reflex actions which otherwise would have occurred be prevented. The observation, *Obs.* 7, where the heating one leg prevents reflex action in the other, seems to point distinctly to such an explanation."

These " observations " of Mr. Harding were what drew my attention to this subject in the first place. If true they are of extreme importance. If an organ, either empty or full of blood, is to behave in one way when directly heated, and in another way, exactly the reverse of the former, when blood heated in a remote part is passed through it, it is certainly a very surprising fact, well worthy of thorough investigation. I have not found, however, any other reference to Mr. Harding's work, and am

forced to believe that he pursued it no further. I am also in the dark as to his exact method of experimentation, but I assume that his frogs were hung up by the jaw and the legs only were immersed in water, as seems to be implied in the passage just quoted ; if they were not, it is possible that some of the remarks I am about to make may be irrelevant.

The results of Mr. Harding's work which have given Dr. Foster a " clue " seemed so novel that I set to work to see if some explanation of the facts could be obtained which would not compel us to believe that heating of the frog's heart from the inside by blood warmed in a remote part has an effect upon it diametrically opposed to the effects of heat directly applied from the outside. I began by making preliminary experiments, and employing the method that I suppose Harding to have used, viz. hanging the frog by the jaw after destroying the brain and spinal cord. The heart was exposed by a small hole cut in the chest wall, and I, like Harding, saw that the heart beat slower as the temperature of the water about the legs in the vessel below, rose.

Bearing in mind the work of Cyon,[20] who has shown that passing hot blood through the mammalian brain slows the heart beat by stimulation of the vagus, I was led to inquire if it might not be that in the experiments of Harding, the hot circulating blood acting as a common heat stimulus, irritated directly the trunk of the vagus somewhere along its course, and so overcame (by ordinary vagus inhibition) that increase of function which the heated blood might be supposed to induce in the heart itself. It was but a forlorn hope ; for aside from the fact that the hot blood pouring through the cavities of the organ would be presumably the more powerful stimulus, it might also be expected that the vagus would soon get wearied ; though between the two antagonizing forces we should look for intermittent or irregular pulsations—which we never get. Still it was possible, and so I tested the idea by making another experiment, after previous administration of a small dose of atropine sulphate. This doubtless paralyzed the vagus, but the result of the experiment was exactly the same as before : the heart beat steadily slower as the water about the legs grew warmer.

In repeating Harding's experiments with the frog suspended by the jaw and his legs in heated water, I was struck, however,

with the emptiness of the heart. Its paleness and feeble beat were conspicuous; the aortic arches were white and empty, while the vessels of the thighs seemed gorged with blood. To the eye there appeared to be little or no circulation, and I was thus led to ask: *Does the blood in these cases really circulate so as to heat the heart?*

If we reflect upon the conditions we must admit that they are highly unfavorable for a good circulation. The brain and spinal cord having been destroyed, all vasomotor centres are out of the question, and their influence in maintaining blood pressure is lost; hence "resistance" is removed, arterial pressure falls, and the blood flows freely from the heart and arteries into the veins; here it settles slowly into the legs and viscera, and remains there (respirations and movement—the conditions requisite for adequate venous pressure—having long since ceased) under the simple influence of gravity. If the web be examined with a microscope, no circulation will be detected in a frog destitute of spinal cord and hanging by the jaw. I can scarcely suppose that Dr. Foster and Mr. Harding have overlooked so elementary a fact, if indeed their experiments were conducted in this way; but the heart certainly does beat slower in these cases, while the legs are gradually heated, though, contrary to Mr. Harding's belief, no hot blood passes through it. A thermometer placed upon the heart or among the viscera, or in the stomach near by, if the heart goes slower, never shows any rise of temperature, though the temperature of the water about the legs may be raised from 20° to 40° while the observation is being made; conversely, if in any such case the heart does beat faster it will always be found to be warmer than before.

Moreover, quite aside from temperature changes, I have repeatedly seen the heart-beat, in a frog destitute of brain and spinal cord, fall as much as from forty-four to twenty-eight beats per minute on simply changing the position of the animal from the horizontal to the vertical. In short, from numerous experiments I am forced to conclude that in cases similar to those described by Dr. Foster as observed by Mr. Harding, the heart beats slower, not because of heat nor from heated blood, but owing perhaps to starvation; possibly to a zero, or even negative venous pressure; or to some cause equally remote. In every

case where the heart was actually heated by warm blood its beats were increased in frequency, often to a surprising extent.

It must not be forgotten that we have, in the vessels of the frog destitute of brain and spinal cord, a system of flaccid tubes, only partially filled with a fluid which, with little or no hindrance, obeys the laws of gravity. It will be found that with no vital spinal cord, the frog's heart behaves very differently according as the animal's body is

    (a) *horizontal;*

    (b) *vertical, with head highest;* or,

    (c) *vertical, with feet uppermost.*

The heart of a frog lying horizontal in a pan of water, and having the spinal cord intact, beats regularly and powerfully, driving into the arteries with considerable energy the blood which goes to keep up the head of arterial pressure. In the frog destitute of a spinal cord, however, it will often be found—particularly if care has been taken to destroy all of the cord—that the arches springing from the base of the heart are white and empty; while if the beats be counted, it will frequently, indeed usually, appear that the rate per minute is growing less and less. Now, the truth is that in this case there is little or no circulation. The heart is really the highest organ in the prostrate flaccid body; and on the familiar principle that liquids will not freely run up hill, the venous blood subjected to no *vis a tergo* in the muscles, and free from the pull of thoracic aspiration, lags behind and gravitates into the lowest veins. If, perchance, any venous blood gets crowded up through the auricles and into the ventricle, it is speedily pumped down the hill again through the arteries, and by their elasticity is driven on into the veins.

I have repeatedly seen cases like the following, which may serve as a type; it is an actual observation.

A small bull frog had his medulla divided and his brain destroyed at 10 A. M. He lost very little blood and rested quietly in a pan of water until just before the beginning of the observations, when the heart was exposed (but left in the pericardium) by a small hole made in the ventral chest-wall. Observations were made once in three minutes.

No. of Observation.	Time.	Rate of Heart Beat per ¼ minute.	Remarks.
1	11.28	35	Arches very red and full.
2	11.31	34	
3	11.34	34	*Bulbus arteriosus* beating
4	11.37	33	very powerfully.
5	11.40	34	
6	11.43	34	Spinal cord destroyed at
7	11.46	39	11.44. Arches paler.
8	11.49	31	
9	11.52	27	Arches white and appar-
10	11.55	23	ently empty.
11	11.58	23	
12	12.01	22	Hung up by the feet.
13	12.04	22	
14	12.07	23	
15	12	24	Arches very full.
16	12.13	25	
17	12.16	24	
18	12.19	24	

(*b.*) *With the body vertical and the head highest,* the condition of things just described is aggravated. The fore part of the body becomes exsanguinated, and even the ventricle, which, in the case last mentioned, usually contains some small amount of blood, may get to be perfectly pale and white. The blood settles away into the hind legs and visceral veins, the great capacity of which is well known. It may be of interest to recall in this connection the fact that after the administration of certain drugs (*e. g.* quinine) which depress the reflex-excitability of the cord and hence impair the circulation, the blood will in the same way be found after a time chiefly in the hind legs and viscera.

Whether the downward pull of the fluid due to gravity may or may not cause a negative pressure in the heart I have not ascertained. When the legs are gently heated in water of a rising temperature the vessels probably relax somewhat, thus further robbing the heart of blood; at any rate we may take it as certain that in these cases the heart is not filled with warmed blood coming from the legs, and its retarded beating cannot be considered as due to that cause.

(*c.*) *With the body vertical and the feet uppermost,* the blood previously contained largely in the hind legs and viscera flows freely down into the heart. This organ fills, gets very red, and beats much more

powerfully. The beats seem also to become more frequent, though the original rate is seldom or never attained.

No better demonstration of the meaning of "resistance" as an element in blood-pressure, or of the fact that this resistance is due to nervous influences residing chiefly in the cord, could be desired for laboratory use. The frog which has been made to pass through stages (*a*) and (*b*), can be turned with now the head and now the feet highest; and the demonstration is complete of a system of partially-filled tubes through which blood flows most freely, and which in spite of an active pump and abundant arterial elasticity is nevertheless, without "resistance," no circulation at all.

From the foregoing considerations it seems clear that if my observations are correct, the "clue" of Dr. Foster leads to nothing. As an "analogy" supporting the theory under consideration it is worse than useless, for it leads to results which tend to weaken that theory. It is plain that the heart of the frog has never yet, when freed from all extrinsic nervous influences, been made by heat or by heated blood to beat at first more slowly; on the contrary it always beats faster when fed with heated blood.

The theory that any organ or tissue having a protoplasmic basis may so far depart from obeying the laws of protoplasm as to reverse them completely, and under gentle heating may suffer loss of functional power with no preliminary phase of increased activity, if true in the case of the spinal cord of the frog (which is plainly protoplasmic), stands now wholly unique, and must be proven beyond all question if it is to stand at all.

The examination of the evidence for and against this theory will be reserved for the second paper.

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NOTES ON THE DEVELOPMENT OF PANOPÆUS SAYI (Smith). By E. A. BIRGE, Ph. D., Professor of Zoology in the University of Wisconsin. With plates XXX, XXXI, XXXII and XXXIII.

The observations on which the following paper is based were made at the Johns Hopkins Summer Laboratory in the summer of 1878. The paper was written in the following college year 1878-9, and was lost in transmission to Baltimore. Absence from the country and press of other work have deferred the reproduction of the paper until this late date, when it seems best to print only such parts as are directly concerned with observations, leaving out all general considerations.

To Dr. W. K. Brooks, the director of the laboratory, I have to offer my thanks for the generous opportunities for study furnished by the laboratory under his charge.

Panopæus sayi (Smith), and *P. depressus* (Smith), are both very common in the neighborhood of Crisfield, Md. They swarm along the muddy shores, under stones and oyster-shells, and, especially *P. depressus*, in the interior of sponges in deeper water.

In spite of this abundance of material I found it impossible to raise any one crab from the egg to the adult stage. Specimens were raised from the egg to the second zoëa stage, and the moults observed from stage to stage throughout. As, however, many moults do not alter the form of the zoëa, it has been found impossible to determine the number of these operations during the larval life.

Egg-development.—As my plan of study did not include observations on the intraovular development of the crustacea, few notes were made on the eggs during the first part of my stay at Crisfield. When I discovered to which crab my zoëas belonged, it was too late to trace in order the development of a single set of

eggs, and those crabs which I left behind in different stages of development unfortunately died. I can therefore present only detached notes on this part of the life-history.

The diameter of the egg is about 0.2 inch. The yolk is composed of olive-green globules of various sizes. The nauplius stage was the first observed (Plate XXX, Figs. 1 and 2). The head-and tail-folds first appear, and, within a few hours, the first three pairs of appendages in rapid succession from before backward. At the same time the telson becomes divided and the labrum is marked off from the head. The branch of the antenna soon appears in the form of a lobe on its posterior side. Both antennae are from the first directed toward the dorsal side of the embryo, and in their growth soon cover the mandible (Plate XXX, Figs. 1 and 2).

In the next stage all the appendages of the young zoëa are present. They rapidly appear—the entire change from Fig. 1 to Fig. 3 (Plate XXX) taking place in less than 18 hours. The antennule has become wrinkled, showing its rapid growth in length; the antenna has gained four small points on the main stem, the rudiments of the four great lobes of the larval skin. The mandible is larger, otherwise unchanged. The first maxilla shows traces of its future lobes, while the second maxilla is bilobed from the start. This last appendage is also crowded out of place and partly concealed below the abdomen. The two maxillipedes are as yet simple outgrowths of the blastoderm, showing no trace of division; their long axis lies parallel to that of the abdomen. The telson shows six lobes on each side, the rudiments of the future cuticular appendages. The clefts separating the head- and tail-folds from the underlying blastoderm are much deeper, and the cephalic lobes are more definite in shape (Fig. 6, intermediate between Figs. 3 and 4).

The next stage is represented by Figs. 4 and 5, Plate XXX. Fig. 5 is from a slightly older embryo than that shown in Fig. 4. Here the larval skin is quite firm and is easily demonstrated. The eye is clearly marked out, though still without pigment. The antennule has its cuticular appendage, and the rudiments of spines and hairs appear on all appendages which are to bear them. The third maxillipede has appeared and is apparently larger proportionally than after hatching, All the maxillipedes

are divided into endopodite and exopodite, and the segmentation of the abdomen is plainly marked. The carapace is present. The yolk still fills the whole dorsal part of the egg.

Development now goes on more slowly. The appendages take on their proper form within the larval skin. The abdomen grows forward between the eyes and reaches nearly to the heart. The larval skin grows out into the long cuticular appendages of the antennule, antenna and telson. The hairs of the maxillipedes develop and are invaginated into the terminal joints of those appendages. Pigment is deposited in the eye and the *macula nigra* appears. Both come at the same time, or the eye a little sooner. This order of appearance reverses that of *Palemonetes vulgaris* as observed by Faxon (*Bull. Mus. Comp. Zool.* Vol. 15, p. 308).

The yolk is absorbed and only a few globules are left at the time of hatching. The rudiments of the spines of the carapace appear.

First Zoëa stage. Still in larval skin.—Plate XXX, Fig. 9, and Plate XXXI, Fig. 1.

When hatched the young Panopæus is still enveloped in the larval skin, which it retains for several hours. The time varies with the activity of the specimen from two or three hours to as many as twenty-four. The shorter times are the more common.

The skin is unsegmented and takes no part in the fold of the carapace, nor is it prolonged for any of the hairs of the appendages or the spines of the telson. It bears, however, numerous hairs itself, and has peculiar prolongations, which will be spoken of in detail under the description of the appendages.

The dorsal, lateral and frontal spines can be detected under the skin, and these, as well as the long hairs of the maxillipede, are ready to push their way out as soon as the larval skin is cast off—or rather in the act of moulting. The same is true of the invaginated antenna and the spines of the telson. The abdominal spines are merely indicated. Numerous spots of black pigment are present in carapace, abdomen, mandible and maxillipedes. The labrum is enormous, projecting downward between the mandibles. No trace of the third maxillipede was

seen, although it is probably present. The thoracic legs have not appeared.

The muscles of the animal are still weak, as is also the skeleton. The animal is sluggish in its movements, and usually carries the abdomen bent, as in Fig. 1, Plate XXXI.

Second Zoëa stage.—Plate XXXI, Fig. 2.

With the casting of the larval skin the regular zoëa form is assumed. It is characterized chiefly by the great length of the dorsal, and, especially, the frontal spines, and by the corresponding length of the antennæ, a feature in which according to Faxon (*Bull. Mus. Comp. Zool.* Vol. VI, No. 10) this species stands alone. The structure is shared, however, by the sister species *P. depressus*, although neither spine nor antenna is so long (*cf.* Plate XXXII, Fig. 12). The maxillipedes bear four long, jointed, plumose hairs on the exopodite. The abdomen has four joints besides the telson, of which the first has a short spine on each side, which bears against the sides of the carapace when the abdomen is flexed. The telson is developed into a long fork, bearing on the inner side six spines, and one on the outside of each arm. The animal is very active, swimming and kicking vigorously.

The beautiful figure of Faxon (*Bull. Mus. Comp. Zool.* Vol. VI. No. 10, Plate II, Fig. 4) represents this stage.

Third Zoëa stage.—Plate XXXI, Fig. 3.

The zoëa moults a large number of times, some moultings making little or no change of form. The stage represented in Plate XXXII is reached after as many as three moults. I have kept a zoëa which moulted twice from the first stage without causing any change of form.

The third stage is characterized by a greater size, greater proportional length of the frontal spine, which may be slightly longer than the antennæ. The last abdominal segment before the telson has developed two long spines, and the spines on the second segment are larger. The maxillipedes bear six swimming hairs, instead of four, and there are thick-set hairs on the edge of the carapace. The abdominal legs can now be distinguished as masses of cells lying under the skin. They cause no elevations of

the skin as yet. The eye is larger and more movable. The thoracic feet are unchanged.

Later Zoëa stages.

Numerous changes of minor importance occur in the moultings between the third stage and the last. The abdominal legs appear, first as simple elevations, then becoming divided by a joint and gaining a rudimentary endopodite. The sense-hairs of the antennule increase in number to six or seven, and the swimming hairs of the maxillipedes to eight and nine respectively. The rudiment of the permanent antenna appears as a small lobe on the inner side of the larval antenna. The number of abdominal spines increases to three pairs.

Last Zoëa stage.—Plate XXXI, Figs. 4 and 5.

The chief characteristics of this stage, apart from greater size, &c., are the division of the telson, the appearance of the mandibular palpus, and the segmentation of the antennule.

The abdominal feet have grown and the endopodite is plainly marked (Plate XXXIII, Fig. 8'). The swimming hairs have increased to twelve or fourteen on each maxillipede. The animal is, however, very sluggish, lying for hours quiet at the bottom of the jar. This habit makes the zoëas of this stage rather rare in the open water, so that it is easier to raise them than to find them. They moult less frequently and accumulate all sorts of *débris* and parasites upon their shells, making their study more difficult.

From the antenna has grown the projection in which the permanent antenna is developed. The outgrowth holds the same relation to the permanent antenna that the larval skin holds, *i. e.* it is a mere sheath—unsegmented. Inside of this sheath the segmentation of the true antenna goes on. The antennule, on the contrary, becomes segmented into three or four joints, and develops a small outgrowth on the basal joint.

A new abdominal segment is formed by the separation of the anterior part of the telson, whose forks are now at their maximum size. A small unjointed palpus appears on the mandible, the thoracic legs are developing inside their skins, and the gills

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[1] From an earlier stage, but essentially like this.

and epipodites are present. It is worthy of note that all the appendages of the crab appear as unjointed projections of the skin, inside of which the segmented appendage develops. In the case of the thoracic legs this is especially marked. Several successive sacs are formed for the developing leg, all unjointed even where joints are distinguishable inside the sac. Joints, however, appear before the zoëa stage is left.

Further peculiarities of the appendages will be considered under the appropriate head.

First Megalops stage.—Plate XXXI, Figs. 7 and 8.

With the moult from the last zoëa stage to the first megalops an enormous change takes place both in the form of the body and of appendages. All of the long spines are entirely lost and leave no trace behind. Panopæus thus differs from Cancer as figured by Smith (*U. S. Fish Com. Rep.*, '71–2, Plate VIII), where the frontal and dorsal spines persist in the megalops. The form of the carapace is changed from one horizontally compressed to one vertically flattened. The abdomen suffers the same change in proportion, and the telson loses its fork and becomes a simple plate. No less marked is the change in the appendages. These will be spoken of in detail later. All are profoundly modified. The maxillipedes and thoracic legs undergo the greatest change, the former losing greatly in size and the latter gaining. The abdominal legs get their hairs. The abdomen is usually carried stretched straight out or slightly bent down, and is used in locomotion. The ear-sac can be seen in the base of the antennule, and the permanent antenna replaces that of the zoëa. Both are partly concealed by a broad flat plate, projecting forward on the carapace. In the middle of this a small notched projection is the only suggestion of the frontal spine. The animal is covered by scattered coarse hairs.

Subsequent changes in the megalops affect the proportions of the carapace, which becomes broader proportionally, and that of the abdomen which becomes smaller, and is permanently flexed under the sternum. The appendages undergo many changes, gradually approximating them to the adult form.

The last megalops stage is reached after several—at least four—moultings.

Leaving this stage the megalops assumes the form shown in Plate XXXI, Figs. 9, 10, the *first crab stage.* Here the carapace has lost most of the broad notched projecting plate in front, and its edge has assumed a curve not greatly different from the adult form. Each side of the carapace bears three teeth which persist in the adult. The abdomen is also nearer the adult form, while the appendages have not greatly altered from the last megalops stage. No specimen was reared beyond this stage.

The youngest crab found is figured in Plate XXXI, Fig. 6. It has already the adult proportions. The crenulation of the anterior border of the carapace is more distinct than later. The outline of the carapace of a large male is figured in Plate XXXI, Fig. 11.

Eye.—First zoëa stage, Plate XXX, Fig. 9; XXXI, Fig. 1. Second zoëa stage, Plate XXXII, Fig. 1. First megalops stage, Plate XXXII, Fig. 2. Adult, Plate XXXII, Fig. 3.

The eye undergoes few changes during the zoëa stage. It becomes larger and more movable as development progresses, but in form and proportion alters little. It is, however, longer in the older zoëas. The eye has the same general form in the megalops stage and through the first crab stage. When it becomes divided into two joints, and when the sinus in the cornea is developed, I cannot say. The adult form is present in the crab of Plate XXXI, Fig. 6.

Antennule.—First zoëa, Plate XXXII, Fig. 4. Second zoëa, Plate XXXII, Fig. 5. Third zoëa, Plate XXXII, Fig. 6. Later zoëa, Plate XXXII, Fig. 7. Last zoëa, Plate XXXII, Fig. 8. First megalops, Plate XXXII, Fig. 9. Adult, Plate XXXII, Fig. 10.

The antennule in the first zoëa stage is enveloped in the larval skin, which extends out in a very long plumose expansion, and bears on one side a short and slender branch. This carries a tuft of hairs at its end. Into this branch the sense-hairs of the permanent zoëa antennule extend. The long projection of the larval skin is plainly homologous to the seta of the antennule of the larval *Callianassa,* as figured by Claus,[1] although that zoëa is in a later stage of development.

[1] Untersuchungen zur Genealogischen Grundlage des Crustaceen System, T. viii, Fig. 2.

In the second zoëa the antennule has the ordinary elongated conical form, and bears one short and two long sense-hairs. In later stages the number of hairs increases to three and finally to six or seven. A lobe also appears on the inside of the antennule.

In the last zoëa stage the antennule has divided into three (? four) joints, and the lobe is attached to the second from the base. One hair among the sense-hairs seems much stouter than the rest, but its subsequent fate was not traced. The number of sense-hairs is greatly increased.

In the first megalops stage the lobe has formed a distinct joint, bearing two hairs on its end. The terminal joint shows traces of segmentation which afterwards disappear. The basal joint is enlarged for the ear.

In the adult the joint formed from the lobe is divided into six parts, and the expansion of the former terminal joint is smaller, although its hairs have greatly increased. The basal joint is also larger.

Antenna.—First zoëa, Plate XXXII, Fig. 11; Plate XXXI, Fig. 1. Second zoëa, Plate XXXII, Fig. 12. Later zoëa, Plate XXXII, Fig. 14. Last zoëa, Plate XXXII, Fig. 15. First megalops, Plate XXXII, Fig. 16. Adult, Plate XXXII, Fig. 17.

The larval skin enveloping the antenna is much shorter than the permanent organ of the zoëa, and bears on one side a very large four-lobed appendage. Into the base of this projects the minute "squamiform appendage" of the zoëa antenna. The lobes of the cuticular expansion are covered with short fine hairs. The spine which forms the main part of the future antenna is greatly wrinkled and invaginated, so as to be only about one-third as long as in the next stage.

The second zoëa stage shows the proper zoëa antenna—an enormously long spine, smooth and gently curving, extending to the tip of the frontal spine. This is the spine of the ordinary zoëa antenna—the "stachelfortsatz" of Claus, "exopodite" of Balfour. It is probably the epipodite, while the " squamiform appendage "—" ramus exterior " of Claus—is the exopodite, and the permanent adult antenna is clearly the endopodite.

The squamiform appendage—apparently overlooked by Faxon—is a minute joint, situated near the base on the inner side of the antenna, and bearing a single terminal hair.

There is no trace of the adult antenna. This structure appears in the older zoëas after numerous moults, as a small lobe on the inner side of the spine. This extends and increases in size during the later changes of skin, and finally the joints of the megalops antenna can be plainly seen within it.

In the moult to the megalops stage the spine and ramus exterior are lost, and the permanent antenna, consisting of about eleven joints, takes its place. The third or fourth joint from the end, as in *Carcinus maenas*, is enlarged and bears large sense-hairs.

In the adult the antenna has 18 to 20 joints, and the sense-hairs are about equal in size. When the opening of the green gland is formed was not determined.

Mandible.—Second zoëa, Plate XXXII, Fig. 18. Second zoëa, Plate XXXII, Fig. 19. Last zoëa, Plate XXXII, Fig. 20. First zoëa, Plate XXXII, Fig. 21. Young crab, Plate XXXII, Fig. 22. Adult, Plate XXXII, Fig. 23.

The larval skin of the mandible presents no features of especial interest.

In the second zoëa stage the mandible bears at each end two projections. Of these the anterior one at the proximal end serves as the articular point, while to the other is attached the main muscle. Of the two distal projections, the outer—lower—is thinner than the other, which is toothed, and serves as the main instrument in chewing. The axis of the jaw passes through this surface and the articular projection; and the appendage is rotated on this axis by the muscles. As the zoëa becomes older the attachment of the muscle (in Fig. 18) extends further toward the distal end of the appendage.

No marked change in the form of the mandible occurs before the last zoëa stage, when the palpus shows itself as a small evagination on the anterior edge. This feature is diagnostic of the last zoëa. The notch in the posterior side, in which the labium lies, becomes deeper.

In the first megalops stage the palpus is three-jointed, and the appendage differs only slightly from the adult form. The two proximal projections are larger proportionally, the cutting surface is less sharp and its tooth is not so clearly marked, the whole structure is broader proportionally. As the carapace grows in breadth the mandible lengthens and acquires the adult form.

The cutting surface of the adult mandible is the lower projection of the zoëa mandible, and the flat surface back of the edge corresponds to the grinding surface of the zoëa.

The upper lip is enormously large in the first zoëa stage, and becomes smaller, covered with hairs and enclosed within the mandibles. The shape is little altered during development.

First Maxilla.—First zoëa, Plate XXXII, Fig. 24. Third zoëa, Plate XXXII, Fig. 25. First megalops, Plate XXXII, Fig. 26. Young crab, Plate XXXII, Fig. 27. Adult, Plate XXXII, Fig. 28.

The larval skin of the first maxilla shows three elevations corresponding to the parts of the appendage. It bears no hairs or setæ.

With the second zoëa stage the regular zoëa maxilla appears. It consists of three parts, of which the outer one is two-jointed. This bears on its basal joint one spine, and five or six on the terminal one. These spines appear to be smooth. Those on the other lobes are bearded with short stiff hairs. There are about six of these stout spines on the middle lobe, and four on the inner.

With the change to the megalops the outer branch is bent proximad and outward and loses most of its hairs. The middle and inner lobes are greatly elongated, and the number of their spines is much increased. Those of the middle lobe are the larger. During the transition from the megalops to the adult the inner lobe becomes curved toward the middle one, and the joint in the outer lobe becomes more distinct than in the early megalops stages.

The sudden outward bend of the outer branch of this appendage at the change to the megalops, recalls the inward bend of the exopodite of the maxillipedes, and suggests a possible homology for the part. The fact that the appendage is bilobed at a very early stage also looks in the same direction.

Second Maxilla.—First zoëa, Plate XXXII, Fig. 29. Second zoëa, Plate XXXII, Fig. 30. Last zoëa, Plate XXXII, Fig. 31. First megalops, Plate XXXII, Fig. 32. Young crab, Plate XXXII, Fig. 33. Adult, Plate XXXII, Fig 34.

The alterations of the second maxilla during the zoëa state are much more considerable than are those of the first maxilla.

In the larval skin at hatching there are four lobes over this appendage, of which the three median correspond to the lobes of the first maxilla.

In the second zoëa stage the appendage has four main divisions. Each of the three median parts is bilobed at the end, and bears from six to eight spines, of which but few are obviously plumose. There is a trace of a joint at the base of the outer of these three lobes. The outer part—the scaphognathite—is the most interesting. This plate is much extended in two directions from the point of attachment. The shorter extension extends distally and outward, and bears four or five long slender projections, hardly to be called hairs. The other and longer projection passes downward, curving toward the median line, bearing very fine hairs on its edges. It is impossible to avoid noting the resemblance of this plate to the epipodites of the adult maxillipedes, especially the first. It forms the entire scaphognathite, and neither at this nor any other time shows a trace of segmentation. Its subsequent changes are merely to fit it in shape to the broadening cavity in which it is to work, and to increase its efficiency by means of hairs on its edge. It is difficult to believe that this plate is composed of epipodite and exopodite united, as asserted by some authors.

The zoëa life causes changes mainly in the scaphognathite, which becomes more oval in shape by shortening its projections, loses its fine hairs, and gains new, long setæ, which become more hair-like and more thickly set.

In the first megalops stage the outer of the three median lobes—the probable exopodite—is a good deal changed. It loses its terminal hairs and becomes fringed with fine hairs on its edges. It no longer shows the terminal lobes, which at one time even hinted at two joints, but is a single slender plate. The other parts are little changed. The scaphognathite is becoming rhomboidal and its hairs are more numerous.

In the first crab stage these hairs have greatly increased in number and are plumose, forming a real extension of the plate so far as work is concerned. The exopodite is also wider at the base.

These features are accentuated in the adult. The exopodite is much broader at the base, the two median lobes are deeply cleft, the scaphognathite is nearly rhomboidal and densely fringed with plumose hairs.

First Maxillipede.—First zoëa, Plate XXXIII, Fig. 1. Second zoëa, Plate XXXIII, Fig. 2. Last zoëa, Plate XXXI, Figs. 1 to 3. First megalops, Plate XXXIII, Fig. 3. Young crab, Plate XXXIII, Fig. 4. Adult, Plate XXXIII, Fig. 5.

In the first zoëa this appendage is closely invested by the larval skin, and the hairs are all more or less invaginated in the joints to which they belong. The hairs are extended during the molt to the second zoëa form; and the exopodite is then furnished with four long, tri-articulate, densely plumose swimming hairs. The endopodite has the normal five joints, each having one hair, except the last, which has several. The long and stout protopodite is covered for its basal half by the carapace. During the zoëa life, few changes take place in this functionally impor-tant appendage, or its fellow, the second maxillipede. The swimming hairs increase in number to six, then eight, and finally twelve. The exopodite in the older zoëas shows marks of a division into two joints.

With the change to the megalops, the appendage greatly alters in form. The epipodite, not seen before, makes its appearance. The exopodite bends abruptly at its middle joint, and the long swimming hairs are much reduced in size. The exact fate of endopodite and protopodite is not clear. They are much reduced and consolidated, and opportunity was lacking to trace the history of each part. Probably the part *a*, Plate XXXIII, Fig. 3, is formed from the two terminal joints of the endopodite, and two or three of the median lobes from the rest of the endopodite, while the protopodite is greatly reduced in size and importance.

The only noteworthy changes in this appendage from the first megalops to the adult form are in the terminal joint of the exopodite, which segments into numerous joints and gains a correspondingly great number of hairs; and in the epipodite, which develops an anterior—lower—lobe homologous to that of the scaphognathite.

Second Maxillipede.—Second zoëa, Plate XXXIII, Fig. 6. Last zoëa, Plate XXXI, Fig. 6. First megalops, Plate XXXIII, Fig. 7. Young crab, Plate XXXIII, Fig. 8. Adult, Plate XXXIII, Fig. 9.

The history of this appendage in the zoëa closely resembles that of the preceding. The main difference is in the endopodite,

which is smaller than that of the first maxillipede, consisting of three joints, of which the terminal one shows in the last stages a trace of division into two parts.

In passing to the first megalops stage the changes of the exopodite are much the same as those of the corresponding part in the next anterior appendage. The endopodite now has five joints, the protopodite has greatly diminished in size, and the epipodite appears.

Third Maxillipede.—Third zoëa, Plate XXXIII, Fig. 14. Late zoëa, Plate XXXIII, Fig. 15. Last zoëa, Plate XXXIII, Fig. 16. First megalops, Plate XXXIII, Fig. 10. Young crab, Plate XXXIII, Fig. 11. Adult, Plate XXXIII, Fig. 13. Comb-hair, Plate XXXIII, Fig. 12.

The third maxillipede appears before hatching as a simple projection, which condition it retains until the later zoëa stages, when the exopodite, epipodite and gill appear as unsegmented projections.

In the first megalops the appendage has a five-jointed endopodite, directed forwards, and the exopodite resembles that of the corresponding stage in the other maxillipedes. The protopodite is not anchylosed to the endopodite. The subsequent changes in the endopodite consist in the enlargement of the two proximal joints, while the terminal then become relatively smaller and bend inward. Finally they become a sort of palpus for the broad plate formed by the basal joints.

In the later megalops stages comb-hairs appear on the terminal joints and are used in cleaning the other mouth appendages.

Walking Legs.—Third zoëa, Plate XXXIII, Fig. 14. Late zoëa, Plate XXXIII, Fig. 15. Last zoëa, Plate XXXIII, Fig. 16. Megalops, Plate XXXI, Fig. 8.

These limbs during the life of the zoëa closely follow the fortunes of the third maxillipede. Like it they first appear as rounded lobes on the sides of the body. At first two appear in the second zoëa (see Plate XXXI, Fig. 3), and no more are present in the third zoëa. In the later stages all are present. The posterior two legs grow forward beneath those already present, and the joints are clearly marked. Gills and epipodites are present.

The legs of the megalops are more slender and joints more cylindrical than are those of the adult. They are sparsely and

evenly covered with coarse hairs, and there is no obvious difference between the right and the left chela.

The segment of the fifth pair of legs is anchylosed to the preceding one at the change from the megalops to the young crab of Plate XXXI, Fig. 8.

Abdominal Appendages.—Fourth zoëa, Plate XXXIII, Fig. 17. Fifth zoëa, Plate XXXIII, Fig. 18. First megalops—Third appendage, Plate XXXIII, Fig. 19. First megalops—Last appendage, Plate XXXIII, Fig. 20. Adult ♀, Third appendage, Plate XXXIII, Fig. 21.

The abdominal legs appear quite early in the larval life. In the third zoëa they may be distinguished as cell-masses below the skin, and in the fourth (with eight swimming hairs) they appear as elevations. They then acquire a small endopodite, and are two-jointed. This condition they retain till the last zoëa, when the hairs are visible, invaginated in the joint. The legs appear first on the fifth abdominal segment, then on the anterior segments, last on the sixth.

In the megalops the exopodite becomes a broad flat plate, which bears from eighteen hairs in the second to six in the last. These are long, tri-articulate and plumose. The endopodite is still a small elevation, and is unjointed.

No series of forms connecting this stage with that of the adult was found. In the adult female the protopodite is much reduced in size, the exopodite much elongated, and the endopodite has six joints.

Characteristics of stages.

First Zoëa.—In larval skin.

Second Zoëa.—Moulted from larval skin, four swimming hairs.

Third Zoëa.—Six swimming hairs. First appearance of abdominal legs under skin. Long spine on fifth abdominal segment.

Fourth Zoëa.—Eight or more swimming hairs. External abdominal legs. Spines on anterior abdominal segments.

Last Zoëa.—Twelve or more swimming hairs. Divided telson. Mandibular palpus.

First Megalops.—Immediately after moult from last zoëa.

First Crab.—Three spines on each side of carapace. Anchylosed segment for fifth walking leg.

Measurements of Panopæus sayi (from single specimens of the stage indicated) given in fractions of an inch:

PART MEASURED.	First Zoëa.	Third Zoëa.	Last Zoëa.	First Megalops.	Young Crab.	Crab of Pl. XXXI, Fig. 6.	Third Zoëa P. depressus.	REMARKS.
Total length..........	.042	.043	.079	.054	.071	..	.047	
" height..........	.01	.063	.106	..	..	..	..	
Carapace length.......	.01	.018	.026	.028	.031	.06	.017	
" breadth.......	.01	.012	.023	.026	.03	.08	.018	Across lateral spines.
" height	.01	.015	.02	.013	..	..	.01	
Breadth between eyes..	.012	.014	.02	.031	.033	.063	..	
Abdomen length......	..	..	.057	.031	.04	..	..	
Frontal spine.........	..	.04	.057	..	..	..	.022	
Dorsal "	..	.017	.022	..	..	..	.011	
Telson length.........	.012	.017	.023	..	..	..	.01	
Antenna	.01	.04	.06	..	..	..	.021	

CUTICULAR APPENDAGES.

Antennule............	.032	..	..	..	..	..	..
Antenna	.033	..	..	..	..	..	..
Telson	.029	..	..	..	..	..	..

PLATE XXXI, Fig. 12, shows the second zoëa of *Panopæus depressus* (Smith), and PLATE XXXII, Fig. 13, its antenna.

The zoëa is readily distinguishable from that of the allied species by the following characteristics:

The spines of the carapace are much shorter proportionally, especially the frontal spine; the antennæ are shorter, more strongly curved, and armed at the tip with short spines; and the telson is much shorter.

Otherwise the zoëas closely resemble each other, and their development is nearly parallel.

The first megalops of *P. depressus* was not found. Nor indeed was there any megalops which could be certainly referred to *P. depressus.* The megalops of *P. sayi* was raised from the zoëa.

October, 1882.

PLATE XXX, Figs. 1–2, Nauplius stage. 3, Stage 2. 4–5, Stage 3. 6, Stage 2, from side. 7–8, Just before hatching.

The yolk is shown only in Figs. 5, 7 and 8.

T = telson, l = labrum, a' = antennule, a'' = antenna, md = mandible, mx' = first maxilla, mx'' = second maxilla, mp' = first maxillipede, mp'' = second maxillipede, e = eye.

Fig. 9.   First zoëa stage from above.

PLATE XXXI, Fig. 1, First zoëa stage. 2, Second zoëa stage. 3, Third zoëa stage. 4, Last zoëa stage. 5, Moult to megalops stage. 6, Young crab (carapace). 7, First megalops stage. 8, First megalops stage. 9, First crab stage (carapace). 10, First crab stage (carapace). 11, Adult crab (carapace). 12, Second zoëa, *P. depressus*.

PLATE XXXII, Fig. 1, Eye, second zoëa; 2, Eye, last zoëa; 3, Eye, adult. 4, Antennule, first zoëa; 5, Antennule, second zoëa; 6, Antennule, third zoëa; 7, Antennule, late zoëa; 8, Antennule, last zoëa; 9, Antennule, first megalops; 10, Antennule, adult. 11, Antenna, first zoëa; 12, Antenna, second zoëa; 13, Antenna, second zoëa, *P. depressus*; 14, Antenna, late zoëa; 15, Antenna, last zoëa; 16, Antenna, first megalops; 17, Antenna, adult. 18, Mandible, second zoëa; 19, Mandible, third zoëa; 20, Mandible, last zoëa; 21, Mandible, first megalops; 22, Mandible, late megalops; 23, Mandible, adult. 24, First Maxilla, first zoëa; 25, First Maxilla, second zoëa; 26, First Maxilla, first megalops; 27, First Maxilla, first crab; 28, First Maxilla, adult. 29, Second Maxilla, first zoëa; 30, Second Maxilla, second zoëa; 31, Second Maxilla, last zoëa; 32, Second Maxilla, first megalops; 33, Second Maxilla, young crab; 34, Second Maxilla, adult.

PLATE XXXIII, Fig. 1, First Maxillipede, first zoëa; 2, First Maxillipede, second zoëa; 3, First Maxillipede, first megalops; 4, First Maxillipede, first crab; 5, First Maxillipede, adult. 6, Second Maxillipede, second zoëa; 7, Second Maxillipede, first megalops; 8, Second Maxillipede, first crab; 9, Second Maxillipede, adult. 10, Third Maxillipede, first megalops; 11, Third Maxillipede, first crab; 12, Third Maxillipede, comb-hair; 13, Third Maxillipede, adult. 14, Thoracic Legs, third zoëa; 15, Thoracic Legs, late zoëa; 16, Thoracic Legs, last zoëa. 17, Abdominal Leg, fourth zoëa; 18, Abdominal Leg, late zoëa. 19, Third Abdominal Leg, first megalops. 20, Last Abdominal Leg, first megalops. 21, Third Abdominal Leg, adult ♀.

STRUCTURE AND GROWTH OF THE SHELL OF THE OYSTER. By HENRY L. OSBORN, Late Fellow in Biology of the Johns Hopkins University. With Plate XXXIV.

All modern accounts of the formation of the Lamellibranch shell accord well with the statement of Huxley that "the shell itself consists of superimposed lamellæ of organic matter hardened by a deposit of calcareous salts. It is a cuticular excretion from the surface of the mantle and never presents any cellular structure."[1]

Dr. Wm. B. Carpenter in 1844 published in the "British Association Reports" a full account of the structure of adult shells in many mollusca. He did not study the development of the shell, but gave it as his opinion, based upon inference from adult structure, that the lime prisms are internal casts of prismatic cells, these cells being layers of cuticle, stripped from time to time from the surface of the mantle. This view of Dr. Carpenter's is taught by Siebold in his Anatomy of Invertebrata,[2] and Bronn leaves the matter an open question, but so far as I can learn the current view is the one quoted above from Huxley.

Since the history of the shell's growth in Lamellibranchs does not seem to have been directly studied by any one, Dr. Brooks suggested last summer at the Beaufort laboratory that I should work upon it; proposing a modification of the method long ago in vogue among the Chinese for growing images of their gods inside the shell of the pearl oyster. He supposed that the study would only confirm general opinion upon the subject, but that observations would be valuable.

The method used was this: the edge of the shell was snipped away with a pair of bone forceps until a gap was produced wide enough to permit the insertion of a thin circular glass cover be-

[1] Anat. Invert. p. 406.
[2] P. 191, edition 1854. Bronn: Classen und Ordnung, III. I Abtheil. p. 346.

tween the outside of the mantle and the inside of the shell. This cover was carefully pushed well back from the gap—it could be done with no appreciable injury to the mantle surface. From their abundance, oysters were at first used. Several of them were taken from the flats, where they grow in enormous numbers, and were provided with glass slips; they were then placed inside a strong, fine wire-net cage and replaced upon the flats. By this means the natural conditions were very nearly obtained, and the protection of the oyster from the army of predaceous crustacea was secured. Under these conditions the oysters apparently went on thriving, and I could from time to time open individuals and learn what had taken place. Studies upon other forms beside the oyster were attempted, but these were not successful.

Pinna is abundant in the waters where the oyster grows, and I attempted to study it in the same manner as the oyster, but without success, since the presence of the cover seemed to irritate the animal. It is quite free from the shell except at the attachment of the adductor muscle, and always succeeds in scrubbing away the glass cover. Other forms were also tried, *Siliqua* and *Venus*, but the attempts were not successful, apparently from the impossibility of closely imitating their conditions of life.

Examination of the glass slips left twenty-four hours inside the oyster, showed a thin gummy deposit. It formed a faintly yellowish brown film, which had hardly consistence enough to hold together. After treatment with staining reagents, haematoxylin, picrocarmine and eosine, the film would show a faint color, but this was diffused evenly in every part and absolutely no structural characteristics could be observed. In some instances lime crystals were already formed, though sparingly. From the character of this young film it is perfectly apparent that it is a viscid excretion poured out from cells upon the surface of the mantle. If one make vertical sections of the mantle properly hardened, it will be seen that the surface is formed of columnar cells. These stand closely packed and are stained intensely. They are glandular and very full of granules; it is they that pour out this very viscid and very abundant secretion. Surface views, also, of mantle stained with silver nitrate show a close pavement all over the mantle formed by the outer ends of these secreting cells.

The hardly consistent film of twenty-four hours has by forty-eight hours become a tough, leathery membrane. Its color is brown. It already forms a definite envelope about the animal, and has shut in the glass cover between itself and the previously formed shell. It resists all attempt to demonstrate any structure in itself by means of the ordinary histological reagents, and is a structureless cuticular or horny envelope, the organic basis of the shell; it is this which is evident as the epidermis in many shells, and which, as may be shown by treatment with dilute acid, forms the skeleton of all shells. In later growths of the shell this membrane or film waxes, fresh supplies of the gummy excretion being spread over its inner surface continually, so that this surface is never so hard and brittle as the outside may become.

In solution in the gummy excretion there is held calcium carbonate, and this, as the film hardens, crystallizes, and gives rise to the various stony structures to which many shells owe much of their beauty. These crystals take on various forms. In one preparation (Fig. 2) they are flat scales with not very sharply cut edges. They are obscurely hexagonal, have an average diameter of $\frac{1}{2400}$ inch, and fill the membrane as thickly as indicated by the figure. If a film of forty-eight hours be placed in dilute acid (acetic was used in this instance), the lime is completely dissolved away and the spaces occupied by the crystals are plainly seen. Such a film is represented in Fig. 3, it is a beautifully tessellated pavement after treatment with the acid, and shows the more or less hexagonal spaces occupied by the crystals. It seems scarcely doubtful that these spaces were formed by lime crystals. Their resemblance to the cells in decalcified *Pinna* shell is so extremely close that two drawings would look identically the same except in respect to the size of the spaces.

Besides the scaly crystals these regularly formed films of forty-eight hours show many crystals which assume forms represented in Figs. 4 and 5. Some are acicular, tapering away from an oval centre, and these are often united into a large nodule, many having formed about some common nucleus. In the figures, which are accurate camera lucida drawings, these crystals are seen to have not as yet formed a continuous layer, and the membrane, being perfectly structureless and almost transparent, cannot be shown. These acicular crystals are generally about $\frac{1}{1800}$ of an inch in

length. They are, however, much less numerous than a second
form (Fig. 5), which is perhaps built upon them. These are ob-
long crystals somewhat swollen at either end and slashed into
many fine points, suggesting striated epithelium cells in the ani-
mal body. These are often compounded into twin and higher sys-
tems, and are frequently seen forming large spiny-looking masses.
They occur in other parts of the same film in which the acicular
crystals may be found, and seem to be the most common condi-
tion of the film after forty-eight hours' growth.

Another film, six days old, has almost completely lost its
leathery character and become stony, from the great amount of
lime present in it. The most of this layer is a thick pavement
of flat cells so closely packed that they are perfectly continuous
over an area of a square inch or more, with here and there small
breaks where the shelly formation has not gone on as regularly.
In these places one sees such crystals as are shown in Fig. 5, but
they are not numerous, also crystals of the sort figured in Fig. 6.
These seem to have a core, which is striated lengthwise, or, as
they finally broaden out at the tip, radially, surrounded by an
outer shell in which the same radially striated appearance is
very strongly marked. These are not common and I hardly
think they can be normal. The size of these nodules is as fol-
lows, viz. in one marked a, greatest diameter of central core
$\frac{1}{500}$ inch, diameter of the peripheral part a little less than $\frac{1}{2400}$
inch. These nodules are thus very much larger than the average
scales of the 48 hours' film whose diameter may be placed at
$\frac{1}{2400}$ inch. They are, however, only about twice the size of the
average scales which make up the bulk of the film at this time.
Dr. Brooks informs me that he has found nodules almost exactly
like these in the shells of *Mya*. It may be noticed that the
peripheral columnar layer bears a very close resemblance to the
prismatic layer as figured by Pagenstecher[1] in his study of the for-
mation of pearls.

I have no studies of the oyster shell later than films of one week
old until we reach films of three or four weeks. By this time
the glass cover is completely shut into the stony shell, and can
no longer be seen, and its place is only to be traced by its form,

[1] Zeitschr. f. wiss. Zoöl. IX, p. 496, plate XX, 1858.

preserved perfectly upon the inner surface of the shell. By breaking out this cover very carefully it is seen to be coated with a thick plate of white shell, which is beautifully smooth upon the side nearest the cover slip. Examination shows this plate to be made up of many lime scales not arranged in any definite system, but with the many layers laid on quite at random. It is of such crystals as these that the bulk of the oyster shell is formed. The inner layer of the shell, or as it is called in Bronn's account, the mother of-pearl layer, forms most of the stony shell, the prismatic layer is almost entirely absent. It is to be regretted, so far as concerns the present purpose, that this is the case, for the oyster is such a quiet animal that the prismatic layer could be readily studied in it were this layer developed in any such beautiful manner as it is in *Pinna*, while *Pinna*, so far as I was able to experiment, did not make a favorable subject.

Upon edges of the oyster shell elongated cells may be seen placed very obliquely; these may represent the prisms of the shells where a prismatic layer is strongly developed. These cells, however, shade off directly into cells which form a close pavement like those of Fig. 3, and seem to be undoubtedly formed in a manner similar to the ordinary polygonal cells of my forty-eight hours' films.

There can be no doubt, I think, on these observations that the shell is formed by the crystallization of the lime in the chitinous sheet as has been generally supposed, and that the older view, that the forms assumed by the lime show that it has been laid down as internal casts, is not at all sustained by the facts in the history of the shell's growth.

It is worth while to mention here a few observations upon young growing oysters as illustrating the wonderful rapidity with which the shell increases. Since the wire cage, in which the oysters were confined and protected, was placed among the growing oysters upon their native flats, it will be seen that not only a favorable place was afforded for the embryonic oyster to attach himself and grow unmolested, but enormous numbers of spawn would be likely to be at hand, and the inside of the cage to be well supplied with them. And such was the case. In a month the box, the stones put into it for ballast, and the oysters

themselves, were literally paved with young oysters about the size of an old-time three-cent piece. In two months these had grown so that only about one fourth of the original number now survived, the others having been literally " shoved out," and the survivors now had shells averaging from three-fourths of an inch to an inch in length, strong and solid, and weighing often as much as three or four grammes.

EXPLANATION OF PLATE XXXIV.

Fig. 1.   Glandular epithelium from the outer surface of the mantle, x½ 3D.

Fig. 2.   Lime scales in film of 48 hours, x½ 3E.

Fig. 3.   Decalcified film of 48 hours, x½ 4D.

Fig. 4.   Acicular prisms from film of 48 hours, x½ 3D.

Fig. 5.   Prisms in film of 48 hours, x½ 3E.

Fig. 6.   Peculiar crystals in film of 6 days, x½ 3E.

The figures were all drawn of the size they appeared with the Zeiss oculars and objectives indicated and reduced one-half in the process of their reproduction.

THE NERVOUS SYSTEM OF PORPITA. By H. W. CONN, and H. G. BEYER, M. D., U. S. N. With Plate XXXV.

The discovery of a nervous system among the Cœlenterata has been one of the important results of modern histology. Starting with Kleinenberg's neuro-muscle cells,[1] which later observations have shown to have been wrongly interpreted, many observations upon the subject by excellent histologists have been made, and to-day it is known that a very primitive, and therefore very interesting nervous system exists in many of the Cœlenterates. The brothers Hertwig found and described such a system in Medusae.[2] From their observations they drew some interesting theoretical conclusions as to the origin of the nervous and muscular systems. Later[3] the Actinia were studied by the same histologists with similar results. The Ctenophorae have been found by Chun[4] and again by the Hertwigs[5] to possess the same nervous system, with a central nerve ring and peripheral scattered ganglion cells. More recently the Hydroids have been the object of special investigation in this regard. Jickeli[6] found in Endendrium and Hydra certain cells, which he considers as nerve cells, scattered quite widely over the animal. Lendenfeld[7] independently discovered the same cells, and extended his observations to include Campanularia. He also discovered in Campanularia what he considers as a central nervous system, in the form of an endodermal nerve ring around the proboscis inside the oral opening.

Our knowledge of the nervous system of Siphonophores is nearly all contained in a short article by Chun[8] upon Vellela.

[1] Kleinenberg. Hydra. Leipzig.
[2] O. and R. Hertwig. Medusen. Leipzig.
[3] Hertwig. Actinia. Jenaisches Zeit. vol. 13.
[4] Chun, Monograph on Ctenophorae of the Gulf of Naples.
[5] Hertwig. Ctenophorae. Jenaisches Zeit. vol. 14.
[6] Jickeli. Morph. Jahrb. vol. VIII.
[7] Lendenfeld. Zool. Anz. No. 131.
[8] Chun. Nervensystem des Siphonophores, Zool. Anz. No. 77.

This paper describes a system of ganglion cells in the ectoderm of Vellela, scattered quite abundantly over nearly all parts of the animal. No central system or nerve ring such as appears in most Cœlenterates was seen. This observation, as far as I am aware, stands alone, but as Chun is a very careful workman there is no doubt as to its truth. Some work which has been done in the Biological laboratory during the present year, upon Porpita, shows that here also is found a similar system of nerve ganglion cells. The observations were made without a previous knowledge of Chun's paper, and are therefore more valuable as confirming his statement as to the existence of a nervous system among Siphonophora, as well as in extending our knowledge of the relation and distribution of the same.

Our specimens of Porpita were collected at Beaufort, N. C., and were preserved by osmic acid. The animals were placed alive in a very weak solution of osmic acid and allowed to stain for a few minutes. Then after washing they were hardened in alcohol, at first in a weak solution, 50 per cent., then in 70 per cent., 95 per cent., and absolute alcohol. This preserved the tissues in beautiful condition for histological work, staining the cell nuclei and the nerve cells slightly. It was hardly necessary to use any further staining reagents, although to bring out the nuclei of the nerve cells it is best to stain the specimen with hæmatoxylin.

To make the arrangement and distribution of the nervous system intelligible, a few words upon the rough anatomy and histology of Porpita will be necessary. Porpita is a small button-shaped siphonophore, with a diameter varying from half an inch to an inch and a half, and with a thickness, in large specimens, somewhat over a quarter of an inch. Their color is a beautiful greenish blue, and when floating on the water with their long tentacles spread out, they are as handsome a specimen as one wishes to find. At sea they are usually seen floating on the surface of the water in large schools, appearing as a greenish band, comparatively narrow but very long, extending in a straight line for miles. They possess some power of locomotion, but this power is slight, and they float largely at the mercy of the winds and waves.

The upper surface of Porpita is a plain, nearly flat, circle,

which is perforated by numerous openings leading into a series of
air chambers lying directly beneath.   The under surface is more
curved in outline, and is covered by large numbers of zooids,
nutritive, generative and tentacular.   The general anatomy can
be seen from Fig. 1, Plate XXXV, which is a perpendicular radial
section through one-half the animal, *i. e.* from the centre to the
edge of the disk.   The upper half of the disk can be seen to be
occupied by a series of air chambers *A C,* arranged in concentric
circles around the centre, each circle being separated from the
others by circular partitions of chitin, and being further divided
by radial partitions into many smaller chambers.   Each cham-
ber communicates with the exterior in two ways.   First, by an
opening through the upper surface, Fig. 1 *o,* leading directly to
the exterior, and second, by means of a large number of tubular
filaments, the pneumatic filaments, Fig. 1 *pf.*   These pneu-
matic filaments arise from the lower side of the air chambers,
and can be traced from these through the lower half of the disk,
pursuing a more or less complex course.   They finally make
their appearance on the under side of the animal, and can be
seen as long tubular threads, which in great abundance are
wound around the nutritive zooids, Fig. 1 *pf.*

The number of these concentric rings of air chambers varies
very much, but they never reach the edge of the disk.   Outside
the outermost air chamber the disk is prolonged into a thin
flexible velum, Fig. 1 *V.*   This velum is filled by a gelatinous
tissue, and is traversed by numerous branching canals.   It is
very abundantly supplied with circular ectodermal muscles, thus
forming a movable membrane extending around the animal and
giving it some power of motion.

Upon the lower surface of the animal are found the various
forms of zooids.   These consist of three kinds.   (1) One very
large central zooid, Fig. 1 *CZ,* the primary nutritive organ.   (2)
A very great number of smaller nutritive zooids, Fig. 1 *NZ,*
varying much in size from minute buds to large organs, nearly
the size of the central zooid.   They fill the space from the cen-
tral zooid to the base of the tentacles, occupying thus a large
part of the under surface of the animal.   Most of the feeding of the
Porpita is done by these zooids, and they serve also as the origin of
the generative organs, the medusae appearing as buds around their

bases, Fig. 1 *GZ.* (3) External to the feeding zooids are three
or four rows of tentacles, Fig. 1 *T.* Most of these tentacles are
very long, even surpassing in length the diameter of the disk;
and when the animal is floating on the water they are stretched
out as a deep fringe around it. The outer rows are younger
and much shorter, not even reaching the edge of the velum.
They are all movable and highly sensitive, and are armed with
quantities of thread cells, many of which are collected in numer-
ous knob-like batteries, Fig 1 *B.*

The most external layer of cells over the whole of the animal
is an ectodermal epithelial layer. The cells of this layer vary
considerably in different regions. Upon the upper surface of the
disk they are high columnar cells, Figs. 1 and 5 *E,* many of
which, especially near the edge of the velum, are epithelio-mus-
cular cells, Fig. 7. Upon the under side of the velum the cells
are smaller and by no means as high, Fig. 5. The nutritive
zooids are covered with a still smaller layer of cells, and upon
the central zooid they become quite flat. The tentacles finally
reach the extreme, and are covered by a layer of large but thin
scale-like cells, Fig. 1 *T* and Fig. 3. Immediately beneath the
epithelial cells is found a layer of ectodermal muscle fibres. In
the tentacles and the nutritive zooids the ectodermal muscles are
longitudinal. The ectodermal muscles found in the velum, how-
ever, are circular muscles. This system of muscles is much more
highly developed than the endodermal muscles which are found in
the nutritive zooids, and to it seems to be due most of the move-
ments of the animal. In all parts of the body there is devel-
oped just beneath the ectodermal muscle layer a supporting
membrane, Figs. 4 and 5 *Sl.* The thickness of this supporting
layer varies much, it being thin in the tentacles, but very thick
in the central zooid and in the upper part of the disk. Succeed-
ing the supporting layer, as we go toward the interior, are found
in some regions a system of endodermal muscles. Neither the
tentacles nor the velum, where the ectodermal muscles are so
powerful, possess endodermal muscles; but the nutritive zooids,
and particularly the central zooid, have an abundant supply.
They form in all cases a circular system. The innermost layer
of cells is the endoderm, which presents many varieties, according
to the region of the body where it is found and the function it

performs. A very peculiar endoderm cell is found in considerable numbers in the tentacles, of which Fig. 8 is a representation. Each has a quite large body, very clear and perfectly transparent. Toward the interior of the tentacle the cell is prolonged as a highly granular columnar process, ending in a knob in which is contained a large nucleus. Toward the exterior the transparent body is continued as a long seemingly tubular process which reaches to the supporting membrane. These cells are found among other endoderm cells of ordinary form which nearly fill the interior of the tentacle. Elsewhere in the animal the endoderm has cells usually characteristic of this layer.

There are in Porpita two distinct structures which are probably nervous in function. The first consists of scattered ganglion cells widely distributed and quite abundant. The second is a large number of organs around the edge of the velum, which seem to be sensory organs of some kind.

Nerve Ganglion Cells.

If a bit of the tentacle of an osmic acid specimen of Porpita be teased out in glycerine, in such a manner as to flatten the ectoderm without pulling it to pieces, quite a number of different ectodermal structures will be seen. Most prominent will be the longitudinal muscle fibres, which section shows are entirely outside the supporting membrane, and therefore ectodermal. Lying among the muscle fibres and sometimes seen to be connected with them are numerous thread cells. The outlines of the ectodermal cells are also plainly seen, showing them to be large flat cells, each of which contains a prominent nucleus. Careful observation will show another structure much less prominent than those mentioned, faintly stained with osmic acid or picro-carmine or more deeply with haematoxylin. These cells are, as far as can be judged from their histology, true ganglion cells. Fig. 3 is a camera drawing of such a preparation. Muscle fibres and thread cells are omitted, to avoid confusion.

The body of these ganglion cells is very small, smaller indeed than the nuclei of the ectodermal cells. They are only about $\frac{1}{2000}$ of an inch in diameter in ordinary specimens, though sometimes somewhat larger. Once seen, however, they can be readily

found in large numbers. Each cell consists of a small cell body with several long processes, Figs. 2 and 3. In a majority of cases the cell bodies are triangular, Fig. 2 *a*, with a long fibre given off from each angle. Bipolar cells are also frequently seen, though they are much less frequent than the tripolar cells; in these cells the body approaches an oval form, Fig. 2 *b*. In still other cases cells with four processes are seen, Fig. 2 *c*. Occasionally multipolar cells with more than four processes are found, though they are extremely rare. The tripolar cells with a triangular body are much the most common.

The body of the cell at first sight seems to be completely homogeneous, and it is with difficulty that a nucleus can be distinguished. Careful examination of favorable specimens, however, particularly those stained with haematoxylin, shows what is represented in Fig. 2. There is present in each cell a large but faint nucleus, nearly filling the body of the cell, and within this a small bright point, the nucleolus. The cell is very slightly granular and usually appears as a clear, almost hyaline mass, in which can be seen the nucleus as a somewhat dark area, and the nucleolus as a small bright spot, Fig. 2.

The fibres which arise from these cells are, as above stated, usually three in number, though there may be two or four, or occasionally more, given off from each cell. Very thin delicate fibres they are, pursuing a tolerably straight course closely applied to the muscular layer. They all divide more or less into finer branches, and thus the processes from each cell cover quite a considerable area. They are remarkable for their extreme length, and can in favorable preparations be traced as delicate branching fibres for a long distance before they finally disappear in the muscular layer. How much farther they may be continued within this layer it is of course impossible to say. Frequently the fibres from one cell unite with those of other cells, as in Fig. 3, thus putting the different nerve ganglia into communication with each other, and forming to a certain extent a continuous nerve plexus. Many of the fibres, however, do not present any such connection with other fibres, but after branching in a complex manner, finally appear to enter the muscular layer lying beneath them and thus disappear from view. They do not seem to have any connection with the thread cells, which

are found abundantly scattered in the ectoderm, although they are found in the ectoderm of the stalks which bear the thread cell batteries, Fig. 1 *B*. They have, indeed, connection with no structures except the muscles.

These cells are entirely ectodermal structures, as is abundantly proved by section. A cross section of the tentacle, Fig. 4, will indicate this relation. The ganglion cells *G* are seen to lie within the ectodermal cells. Beneath them are the ectodermal muscles *M*, and still further toward the interior is seen the supporting layer, Fig. 4 *Sl*, which separates ectoderm from endoderm. This system of ganglion cells therefore lies in the outermost layer of the ectoderm, even exterior to the ectodermal muscles. The same can be seen in sections from other parts of the animal. Fig. 5 is a section through the edge of the velum, and shows the ganglion cells *G* lying among the ectodermal epithelial cells and outside the ectodermal muscles. All the ganglion cells are ectodermal, therefore, and the endoderm does not seem to possess nervous elements, as is the case in some hydroids[1] and in ctenophorae.[2]

Though the cells are most readily seen in the ectoderm of the tentacles, owing to the thinness of the ectodermal cells in this region, after they are once recognized they can be found in various other parts of the animal, and are indeed quite widely distributed. They are always found in connection with the muscular system, and are most abundant when this system is most highly developed. All of the tentacles are well supplied with them. The velum, which is highly muscular, is particularly rich in its nerve supply, both upon its upper and its under surfaces. The nerve cells are found here more abundantly than elsewhere. Upon the upper surface of the animal, as we approach the centre, the muscular system becomes less and less noticeable, and parallel with its decreasing importance the nerve cells become less abundant. They can be found, however, scattered here and there over the entire dorsal surface of the disk. Upon the nutritive zooids we have been unable to find a single ganglion cell, although we have searched patiently for them. Neither can they be found in the ectoderm of the central

[1] Lendenfeld, Zool. Anz. No. 131.
[2] Hertwig, Ctenophora, Jenaisches Zeit. Vol. XIV.

zooid, although here, owing to the thinness of the ectoderm cells, they would be easily seen if present. Chun, in his paper on Vellela, states that the nerve cells are to be found here as well as elsewhere. This is certainly not the case in Porpita, for in no case, either by teasing or by section, have we been able to discover a single nerve cell in any of the nutritive zooids.

The distribution of the ganglion cells then is as follows : They lie wholly in the ectoderm, and their fibres, after running for a considerable distance beneath the outer ectoderm cells and immediately upon the muscle layer, finally penetrate this layer and are lost. The whole of the upper surface of the animal is supplied with them, somewhat sparsely toward the centre, but much more abundantly toward the edge and especially in the velum. The under surface of the velum has also a rich supply, and the tentacles which come next in order contain large numbers. Beyond the base of the inner row of tentacles, toward the centre of the lower surface, they are no longer to be seen either in the secondary nor the central zooid.

The numbers of ganglion cells in these different regions differ very much, but everywhere, even where they are the most abundant, their relatively small number is quite surprising if they are to be considered as forming a nervous system. In the tentacles, where the ectodermal cells are large, there is found on an average about one nerve cell to a dozen ectodermal cells. In the upper surface of the velum they are somewhat more abundant, but owing to the fact that the ectodermal cells are smaller their relative number is much less; while in the centre of the upper surface not more than a single nerve cell is found to 200 or 300 ectodermal cells. They are not distributed with any regularity. Quite a number may be found lying very near together, Fig. 3, in adjacent or even in the same cell, and then there will be seen a large tract which does not seem to be at all supplied with them. Nothing like a central system can be made out. No union of the cells into a nerve ring, such as has been made out by the Hertwigs[1] in Medusae and by Lendenfeld[2] in Eudenduum, seems to exist.

There is still perhaps some doubt as to whether the structures here described are really what they have been considered ;

[1] Hertwig. *Loc. cit.*   [2] Lendenfeld. *Loc. cit.*

whether they may not be some form of connective tissue cor-
puscle without any nervous function. They are, as we have seen,
very few in numbers as compared with any organs which they
are supposed to enervate; they are connected with no central
system, and simply form a more or less connected plexus of scat-
tered cells. If they are true nerve elements they are only to be
considered as what may be the beginning of a nervous system.
It can hardly be possible that they play any important function
as nervous organs. Always associated as they are with the mus-
cular system, they are to be regarded as muscular rather than
sensory cells; but the relatively small number of even their fibres,
as compared with the number of muscular fibres which each
must be supposed to control, certainly indicates that the mus-
cular system cannot be to any great extent dependent upon
them for its stimulation. The cells here described and those
described by Chun in Vellela are, however, undoubtedly similar
structures to those found by various observers in other Cœlen-
terates, and in many cases, as in Medusae and Actinia, they are
connected with a central nervous system. In these cases there
can be little doubt as to their nervous functions. The fact of
the great resemblance of the cells here found to those of the
peripheral nervous system of other cœlenterates, shows therefore
that we are probably correct in viewing them as nervous struc-
tures, and as forming a very primitive nervous system, but one in
which the nervous function is probably very slightly manifested.

Sensory Organs.

Under this head are included a group of organs, hitherto
undescribed, whose nature is somewhat problematical, but which
from their structure seem to be organs of sense of some kind.

If the velum of Porpita be examined from the upper surface
with a lens, it will be seen that its edge is not a plain circle, but
is marked by serration, and looks somewhat like the rim of a
wheel studded irregularly with small cogs. A close examination
shows that this is due to the presence of a series of organs, many
hundreds in number, which, side by side, are arranged around
the edge of the velum. Each organ is a small ectodermal
pocket, and is separated from its neighbor by a small space,
equal in width perhaps to that of the pockets themselves. They

thus form a sensory ring extending around the edge of the disk and composed of hundreds of entirely separate organs.

The minute structure of these organs can only be made out from sections and teased specimens. They are best seen in radial sections through the edge of the velum. Such a section is shown in Fig. 1 *S*, and much more highly magnified in Fig. 5. Such sections show at a glance the nature of the organs. They are nothing more than little invaginations of the ectoderm, forming a little pocket filled with peculiar cells. The supporting membrane, separating the ectoderm from the endoderm, can be traced along the velum to its edge *Sl*, and there bending increased to form the inner lining of each pocket *Sl'*. Beneath the supporting membrane, in the interior of the velum, is seen a gelatinous tissue perforated by numerous endodermal canals, Fig. 5 *C*. Outside this membrane, upon the upper and under surface of the velum, lie the ordinary ectodermal epithelial cells, and outside the same membrane, but within the pocket formed by its invagination, lie a large number of cells, still ectodermal cells but highly modified.

The cells which fill these pockets are large and highly specialized, but they are nevertheless only modified ectodermal cells. This is readily proved by examination of many sections which show a complete gradation from the ordinary ectodermal epithelial cells to the large peculiar cells in the interior of the pocket, Fig. 5. Toward the edge of the pocket the ectodermal cells of the velum are seen to elongate, and thus, even at the deepest part of the organ, while the base of the cell is applied closely to the supporting membrane, its free end is still upon a level with the rest of the ectodermal cells. The ectoderm cells can thus be traced from the short columnar cells, by almost insensible changes, to the peculiarly modified sense cells in the interior of the sensory organs.

Each pocket of this row is thus seen to be filled with a large number of long, quite large cells, with a broad base applied to the supporting membrane, and with their narrower free ends lying exposed to the exterior. Two distinct types of these cells can be distinguished, although they usually graduate into each other without an abrupt break. There are first in the middle and deepest part of each pocket a number of large cells, very

highly granular, Fig. 5 and Fig. 6 *a*. Each of these cells is
somewhat conical in shape, with its apex, in most cases but not
in all, reaching the surface of the velum and thus exposed to
the water. At its base the cell shows a broad band more highly
granular than the rest, Fig. 6 *a*, in which is seen a very large and
very distinct spherical nucleus containing a prominent nucleolus,
Figs. 5 and 6. These cells fill the middle of each pocket. The
second type of cell is found around the edge of the organ, some-
times passing insensibly into the cells of the first type and some-
times ending more abruptly. They differ from the first type in
being much more slender, and in not being granular, but com-
posed of a clear hyaline substance which appears perfectly homo-
geneous. Each cell shows one or two swellings within which is
an oval mass of more dense material, which stains more deeply
than the rest of the cell. It is the nucleus, but it is seldom
definitely outlined, and in no case is it as prominent and distinct
a structure as is the nucleus of the central cells. No nucleolus
is discernible. These cells are much more abundant than those
of the first type, occurring in thick masses around the sides of
each pocket and enclosing the central cells in the middle. In
their natural position they remind one somewhat of the layers of
rods and cones in the retina of the eye. At the extreme edges
of the organs they of course become shorter and finally pass
into the ordinary ectoderm cells.

The functions of these organs it is impossible to tell with cer-
tainty without observations on living specimens, and as we have
only had alcoholic specimens to work upon, we cannot say con-
clusively what they are. From their histological appearance,
however, they would seem to be organs of touch. The presence
of such long delicate cells with free ends exposed to the sur-
rounding water would certainly point to such a function; and
their position at the extreme edge of the velum would favor the
same view. They have no connection with the nerve ganglia
above described; not a single nerve cell is to be found in them
or in any way connected with them. But this is not surprising,
for we have seen that the ganglion cells are associated with the
muscular system alone, and their absence in these bodies is to be
expected. Until further evidence can be obtained they may be
considered as organs of sense and probably organs of touch.

These same organs degenerate with great readiness.   In specimens kept in aquaria for a few days, the whole of the central cells, except the densely granular area at their base, fused into a homogeneous mass, giving them the appearance of secreting organs.   In well-preserved specimens, however, the cells are distinct and have the above-described shape.

EXPLANATION OF PLATE XXXV.

FIGURE. 1. A diagrammatic perpendicular radial section of Porpita from the centre of the animal to its circumference.

      B. Batteries of thread cells.

      O. Opening of air chambers through the upper surface.

      V. Velum.

  AC. Air chambers.

  CZ. Central nutritive zooid.

  GZ. Generative zooids.

  NZ. Secondary nutritive zooids.

    pf. Pneumatic filaments.

FIGURE 2. Specimens of nerve cells.

    a. Tripolar cell.

    b. Bipolar cell.

    c. Quadripolar cell.

FIGURE 3. Teased preparation from tentacle, showing ectodermal cells and ganglion cells.

    G. Ganglion cells.

    F. Nerve processes from the cells.

FIGURE 4. Cross action of tentacle.

    G. Nerve fibre.

    M. Muscle fibres in section.

    Sl. Supporting membrane.

FIGURE 5. Cross section through edge of velum showing sensory body.

    C. Endodermal canals of velum.

    E. Ectodermal epithelial cells.

    G. Ganglion cells.

    Sl. Supporting membrane.

    Sl'. Supporting membrane lining the sensory organs.

FIGURE 6. Sense cells from sensory organs.

    a. One of the larger central cells.

    b. Smaller peripheral cells of the sense organs.

FIGURE 7. Epithelio-muscular cells from the upper surface of the velum.

FIGURE 8. Peculiar endodermal cell found in the tentacles.

Figures drawn by H. W. Conn.

ON THE PRESENCE OF CILIATED EPITHEL-
IUM IN THE HUMAN KIDNEY. By ALBERT H.
TUTTLE, Professor of Zoölogy in the Ohio State University;
Fellow by Courtesy of the Johns Hopkins University. With
Plate XXXVI.

The presence of vibratile cilia in the renal organs of the cold-
blooded vertebrates was fully established many years ago: the
extent of the observations made in that direction toward the close of
the last half century is, however, not generally recognized. Those
of Bowman (Philosophical Transactions, 1842) upon the kidney
of the frog are most commonly referred to, and are frequently so
cited as to leave the impression that only the neck of the capsule
was known to be ciliated; that author, in the paper referred to,
interested as he was in a far different question, that of the true
relation of the Malpighian corpuscle to the uriniferous tubule,
making mention only of the cilia observed in the neck of the cap-
sule and in that portion of the capsule itself which immediately
adjoins the opening into the tubule. The publication of this
important paper, which, as is well known, contained the first true
solution of the question with which it directly dealt, called the
attention of observers to the organs in question, which were, in
accordance with the usage of the day, very generally examined
in the fresh condition: the fact last mentioned gives the reason
why structures which have to a great extent escaped observation
in the hardened and stained preparations more common at the
present day were seen with the far less efficient instruments of
the earlier observers. Bowman speaks of the cilia as seen in
action, producing a current away from the capsule, beyond the
neck of which he did not follow them. Kölliker, however (*Mül-
ler's Archiv*, 1845), describes cilia in action throughout the entire
extent of the tubules in the kidney of an embryo lizard; and in
a note to Kölliker's paper Müller states that he has observed the
same phenomenon in the tubules of the kidney of a skate. Re-
mak (*Froriep's Neue Notizen*, 1845) records the observation of

cilia in action throughout the extent of the tubules in the kidneys of lizards and newts. G. Johnson, the author of the article on the kidney in Todd's Cyclopedia of Anatomy (Vol. IV, 1848), speaks of ciliary action as observed by him in all portions of the tubule in the kidneys of two genera of newts, Triton and Lissotriton, in considerable portions of the tubules of the kidney of the frog, and through a large extent of the tubules in the kidney of a snake: he also predicts their eventual discovery in the kidneys of all vertebrates. In 1854 Kölliker, in his *Microscopische Anatomie*, mentions the ciliation of the tubules in reptiles, amphibians, and fishes as a well-established fact, referring to the observations cited above and others. This conclusion, while fully recognized by those who have carefully examined the matter, seems to have dropped out of the general literature of the histology of the kidney; the observation of Bowman upon the neck of the capsule being, as I have already said, the only one generally cited.

As regards warm-blooded vertebrates our present knowledge is far less extensive. Most of the papers above alluded to speak of the impossibility of recognizing the cilia in the kidneys of the animals under consideration after their characteristic action had ceased: this doubtless takes place as an almost immediate consequence of the change of temperature caused by the removal of a portion of the kidney of a bird or mammal to the stage of the microscope; and the best microscopes of that day, and indeed of a much later period, were wholly inadequate to the detection and resolution of such delicate and thickly set cilia as are really present, when in a state of rest. Gerlach, however, as quoted by Kölliker (*Micr. Anat.*), saw what he believed to be ciliary action in the kidney of the common fowl, and Hassall (Microscopic Anatomy, London, 1852) described it as witnessed by him in the kidneys of the sheep, the horse, and the rabbit.

The first person to recognize the presence of ciliated epithelium in the hardened and stained mammalian kidney was Klein, who published in the Quarterly Journal of Microscopic Science for April, 1881, a notice of their detection in the kidney of the mouse. He found them in the neck of the capsule, but makes no mention of having seen them in any other portion of the tubule. The object of the present communication is to call attention not only to their presence in the human kidney, but also to their extensive

distribution; and to record similar observations made upon the kidney of the cat.

The human kidneys which I have examined in this connection were obtained from a series of autopsies made during the month of February last at the small-pox hospital by Dr. W. T. Council-man (who was then lecturing on pathological histology in this laboratory), under very favorable circumstances as regards their perfectly fresh condition: they were carefully hardened in alcohol, being intended originally for the demonstration of micrococci. Their exceptionally fine state of preservation led me to study them carefully with high powers, with the result (among others) of the detection of the cilia in question in all that were not extensively diseased, viz. in sixteen out of nineteen kidneys examined.

The sections made use of were from .01 to .03 mm. in thickness, were chiefly stained with Bismarck brown and mounted in glycerin, though some were examined unstained or stained with other reagents, and some were mounted in balsam. It was while studying the structure of the nuclei with a Zeiss one-twelfth oil-immersion objective that I came, to my surprise, upon fine, closely set cilia projecting freely into the lumen of the tubule, which is considerably enlarged in the small-pox kidney. Although they were seen in numerous places in the section under examination, my first impression was that each place under consideration must be in close structural proximity to the classical neck of the capsule of its respective tubule, until after several days' examination of the same section, when I came upon the region represented in Fig. 1, Plate XXXVI, which is plainly the place where the lower part of the convoluted tubule ("spiral portion" of Schachowa) passes into the descending limb of Henle's loop. The subsequent examination of a large number of sections from the whole series of kidneys in my hands has convinced me that the convoluted tubule is very extensively if not generally ciliated. Fig. 2 represents a cross section, and Fig. 3 a longitudinal section of such a tubule. (Figs. 1, 2 and 3 are drawn from different kidneys.)

It is somewhat remarkable that while I have examined an indefinite number of capsules lying in the planes of the sections that I have studied most carefully, I have not happened to come

upon a single one in which the plane of the section coincided with the neck of the capsule. I am therefore as yet unable to say from observation whether or no the cilia exist at that historic point. In the case of the cat, however, I have met with something approximating success in this direction, as I shall presently state.

The question of the relation of the cilia to the rod-like bodies (or *stäbchen*) of Heidenhain readily presented itself. As the alcohol-hardened human kidneys did not reveal these structures, I determined to make a comparison of the two kidneys of some mammal, one hardened with alcohol and the other with some chromium compound. A kitten three or four days old was therefore killed and the kidneys immediately removed, one being divided and placed in strong alcohol, and the other treated in a similar way with Müller's fluid, a solution of ammonium chromate not being on hand.

The kidney of the kitten at this age presents a very interesting functional " waking up " (if I may so term it) from within outwards; the more central of the glomeruli and tubules being fully developed and evidently active, while the more peripheral are still quite embryonic. I hope to consider this further at an early date.

The alcohol-hardened kidney was first examined for cilia: these were readily found in the more active portions of the kidney where the lumen of the tubule was sufficiently large: the smallness or absence of the lumen in the more distally situated tubules made a satisfactory examination impossible. Fig. 4 represents a somewhat longitudinal section of a convoluted tubule from this kidney, the plane of section cutting the lumen of the tubule at two or three adjacent points in the course of the latter. Fig. 5, to which I desire to call particular attention, represents a section passing through a Malpighian corpuscle situated in the zone between the more active and more embryonic portions of the kidney. As I have endeavored to represent, the plane of section passed a little above the neck of the capsule, though nearly parallel to it, a bit of the capsule thus overhanging the opening into the neck. The capsule is lined throughout the greater portion of its extent with the flattened epithelium usually described as characteristic of its whole surface, but as this ap-

proaches the neck it passes rather abruptly into a cuboidal epithelium, which in the portion outlying the overhanging part of the capsule above referred to is plainly seen to be ciliated. We have here cilia within the capsule, the situation in the mammal recalling that figured by Bowman (*loc. cit.*) in the frog's kidney, and by Ecker (*Icones Physiologicœ*, 1851–9) in that of a snake (Tropidonotus).

The kidney hardened in Müller's fluid showed the presence of the rod-like bodies of Heidenhain distinctly, though not conspicuously; and also, though not as clearly as in the case of that hardened in alcohol, the cilia, situated in some cases upon cells in which the former structure could be detected, in others upon those in which it was not demonstrated. I am not prepared to state any definite conclusions as to the relation between the two.

The cilia in the human kidney are from 3.5 to 5 μ. in length, in the kitten somewhat less: they are exceedingly fine and very numerous and closely set; hence the great difficulty of their resolution. I am of the opinion that they will eventually be demonstrated in the kidneys of mammals generally. Where present they may be seen, I think, without difficulty under the following conditions: first, the material should be perfectly fresh; the kidneys should be taken from the body of the animal in question immediately after killing (in the case of the human subject within a very few hours after death) and speedily hardened—preferably, I think, with alcohol—at a low temperature; second, the sections employed must be quite thin; third, they should be lightly stained, if at all, and high-colored staining-fluids, such as carmine and hæmatoxylin, should be avoided; fourth, they should be mounted in glycerin; after one is familiar with the appearance of the cilia they can be recognized in balsam preparations, but with considerable difficulty; finally, the examination of the sections should be made with objectives of high aperture: high amplification is not so important. My own examinations have been chiefly made with a Zeiss one-twelfth, but in part also with a Gundlach one-eighth and a Tolles one-sixth, all so-called homogeneous-immersion objectives; after becoming familiar with my sections I could recognize the *presence* of cilia with water-immersion objectives of various makers, by the detection of what appeared to be a striated layer over the granular cells of the epithelium; no dry objective that I have used has

proved able to resolve this " layer" even into distinct striation, though I can generally recognize its nature by the characteristic diffraction color that is produced.

I have gone at length into the conditions which I believe to be important for the successful observation of cilia in mammalian kidneys, partly with the hope that others may be interested in taking up the search in this direction, and partly for the purpose of throwing light upon observations already made. In this latter connection I would mention a paper in *Virchow's Archiv* for Feb. 2, 1883, by S. A. Lebedeff (*Zur Kenntniss der feineren Veränderungen der Nieren bei der Haemoglobinausscheidung*) ; the "striated border," figured and described by that author in connection with the epithelium. of the convoluted tubule in the kidney of the dog, presents an appearance exceedingly similar to that seen when a layer of cilia (clearly shown as such under a homogeneous-immersion objective) is examined with a good water-immersion objective of moderate aperture.

The general distribution of ciliated epithelium throughout the convoluted tubules of warm- and cold-blooded vertebrates alike, if established, would indicate a corresponding functional importance. The suggestion that the cilia play a considerable part in the propulsion of the urine toward the pelvis of the kidney, is probably the most reasonable.

The figures in the plate were all drawn in outline with the camera lucida upon the same scale, and the details afterwards added. They represent, as nearly as it is in my power to do so, the appearances observed; my want of skill as a draughtsman and my lack of familiarity with the peculiar mode of drawing required by the process of reproduction employed must divide the responsibility for all obvious defects. The cilia are perhaps rendered too conspicuous in all the figures; this is certainly the case in Fig. 1.

DESCRIPTION OF PLATE XXXVI.

Fig. 1.   Union of convoluted tubule (spiral portion of Schachowa) with the descending limb of Henle's loop. Man.

Fig. 2.   Cross section of convoluted tubule. Man.

Fig. 3.   Longitudinal section of convoluted tubule. Man.

Fig. 4.   Longitudinal section of convoluted tubule. Kitten.

Fig. 5.   Malpighian corpuscle, showing ciliated epithelium within the capsule. Kitten.

ON THE EFFECT OF VARIATIONS OF ARTE-RIAL PRESSURE ON THE DURATION OF THE SYSTOLE AND THE DIASTOLE OF THE HEART-BEAT.

By WM. H. HOWELL, A. B., Fellow in Biology, and J. S. ELY, Ph. B. With Plate XXXVII.

That variations of arterial pressure have no direct influence on the rate of beat of the isolated mammalian heart has been clearly demonstrated by the investigations of Professor Martin (¹). It is possible, however, that although the pulse-rate in any given time may remain unchanged, still the duration of the systole or of the diastole in each individual heart-beat may be altered, according as the arterial tension is increased or diminished. A shortening of the systole, for instance, might be compensated by an increase in the length of the diastole, or vice versa, so that the total number of beats in a given period would be unaffected; just as in electrical stimulation of the heart, when a systole is provoked before the completion of the previous diastole there is a compensatory increase in the following diastole, the pulse-rate in a given time remaining the same (²). Since the rate of beat of the heart is not directly affected by variations of arterial pressure, within limits, it follows that any change in the duration of the systole consequent upon a change in arterial pressure must go hand in hand with an inverse change in the duration of the diastole. The same holds true, of course, for any change in the length of the diastole.

In view of the fact that alterations in the time relations of the heart-beat, as the direct result of changes in arterial pressure, might take place, although the pulse-rate remained the same, it seemed well to submit the question to investigation, especially as positive statements with regard to the influence of greater or less arterial resistance upon the time of the systole or diastole are not unfrequently met with in physiological works. Marey (³) considers that it is principally the diastolic phase of the heart-beat which is affected. According to him, when an increased resistance is

opposed to the heart, although the length of the systole itself may not be altered, yet the following diastole will be of greater duration in order that the heart may recover from the excessive effort it has made. Talma ([4]), on the contrary, in a recent article makes the statement that the "duration of a ventricular systole increases as the resistance increases." It is possible that in a heart still in connection with the rest of the body, and especially the central nervous system, the duration of the systole may be indirectly influenced by changes in resistance, but we hope to show that in a heart completely isolated from extraneous nervous influences and cut off from all other organs of the body, except the lungs, variations of arterial pressure alone, within wide limits, have no direct effect upon the systole and diastole with regard to their time relations.

Our experiments were all made upon the isolated heart of the dog, kept alive by feeding with defibrinated calf's blood. The method of isolating the heart has been described by Professor Martin in former numbers of this journal (Vol. II, Nos. 1 and 2). The method used by us is the same in principle, although very much altered in many of its details. To briefly repeat the essential points of the operation : the animal, tied down upon a dog board, is anæsthetized by means of a mixture of chloroform and ether, both carotids are ligated and the vagi cut ; the top of the sternum is removed and the internal mammary arteries ligated ; artificial respiration is, of course, used after this point has been reached. As quickly as possible the sides of the thorax are cut away, a cannula placed in the left subclavian artery, the right subclavian ligated below the origin of the vertebral, and the superior vena cava and azygos vein tied. A large cannula is then placed in the aorta and fastened by a stout ligature just below the origin of the left subclavian ; through this cannula the heart pumps out its blood after being removed to the warm case. A large glass cannula is now introduced into the inferior vena cava below the diaphragm. This cannula is connected by rubber tubing with a Mariotte flask filled with defibrinated and filtered calf's blood heated to 37° C. The air in the tubing and cannula, it is scarcely necessary to say, is replaced by blood before the latter is placed in the vein. The warm blood is now allowed to run into the heart from the flask while the clamp is removed from the left subclavian

artery, and the heart permitted to pump out all coagulable blood through a tube connected with the cannula. Care must be taken at this part of the operation to keep up a good arterial tension by partially clamping the outflow tubing. The coagulable blood is also removed from the aorta through the cannula connected with it. When all of the dog's own blood has been washed out of the heart and lungs, the animal is transferred to the warm case. The arrangements here can be scarcely understood without the aid of a diagram. In papers shortly to be published, embodying the results of some previous work by Professor Martin and others under him, the details of the apparatus with an accompanying diagram will be given. It is sufficient to say that within the case are two large Mariotte flasks, each capable of holding several litres of blood, and so arranged that they can be used alternately, the heart, when receiving blood from one flask, pumps it out through the cannula in the aorta and the long rubber tubing which is now connected with it, back into the other flask, so that when one is empty the other is ready to be used. The tubing connected with the aorta extends above the top of the case, and the arterial pressure against which the heart works can easily be varied to any desired extent by increasing or diminishing the height of the end of this tube above the heart. The exact variations in arterial pressure thus produced are given by a mercury manometer connected with the cannula in the left subclavian artery. The pen of this manometer writes upon the roll of paper of the kymograph, and from its tracings the pulse-rate is also obtained.

The essential point in our experiments was to register accurately the duration of the systole and the diastole of the heart isolated in this way and exposed to varying arterial pressures. It would have been a comparatively easy matter to have taken tracings of the heart-beat directly by means of levers, after the method employed by Hoffa and Ludwig [5], or by the application of the more simple device used by Baxt [6]. But it seemed questionable to us whether such methods possess sufficient accuracy. Outside of the complications arising from the possible changes in position of the lever on the heart's surface, or from changes in position of the heart as a whole, it appears very uncertain whether or not the very beginning of the diastolic relaxation will be promptly

registered by such instruments. Owing to the smallness of the dog's ventricle, on the other hand, it is scarcely practicable to introduce an ampulla into the heart in the way employed by Chauveau and Marey ([7]) for the horse.

The method determined upon, and which, it seems to us, leaves but little to be desired in the way of accuracy, was as follows. After the operation of isolating the heart was finished, and the dog had been transferred to the case, a catheter with terminal and side openings was passed down the superior cava and right auricle into the right ventricle, and fastened firmly in position by a ligature around the superior cava. The catheter was filled beforehand with defibrinated blood. Its free end was connected by means of lead tubing, as short as possible and filled with 0.6 per cent NaCl solution, with an ordinary Fick spring manometer. The arm of the manometer carrying the writing point, had all vibrations of its own, arising from its inertia, dampened in the usual way by a carrier immersed in oil. The tracings were taken upon the blackened paper of a rapidly revolving drum-kymograph, upon which, immediately under the manometer pen, a tuning fork vibrating fifty times a second was likewise made to write. The accuracy of the manometer in recording rapid variations of pressure was tested before using by connecting it with a small rubber bag, filled with liquid, which could be compressed under an ordinary telegraph key, the beginning and end of the stroke being registered by an electro-magnet. The writing point of the manometer did not move in a straight line, but described the arc of a large circle. When the height of the curve was small, not exceeding ten or twelve millimetres, this arc did not differ appreciably from a straight line. In most cases, however, it was necessary to introduce a correction for this error. The correction was made by simply allowing the pen to describe its arc upon the drum when stationary, and then measuring the displacement from the vertical for any given height. The difference was added to or subtracted from the recorded time of the systole, according as the displacement was in the direction of the movement of the drum or opposed to it.

In every case but one the tracings were taken from the right ventricle, owing to the fact that a catheter can be introduced into this side of the heart with great ease and without causing any

injury. We made several attempts to place a catheter in the left ventricle, either through one of the pulmonary veins or through a slit in the left auricular appendage. By the latter method it is necessary to remove the pericardium and to expose the heart to more or less handling. The consequence was that' it never lived well for any length of time after the operation. By the former method we succeeded in obtaining several series of observations, one of which is given in the following table (Experiment VII). The results are in accord with those obtained from the right heart. It can make but little difference from which of the ventricles the tracings are taken, since the complete synchronism of the two sides of the heart, when beating normally, is a matter about which there can be no doubt.

In making an observation tracings were taken simultaneously upon the drum and the large kymograph, beginning generally with a mean arterial pressure. As soon as one tracing was finished the arterial pressure was quickly changed, and another similar tracing taken. So that the heart was not exposed as a rule to any given arterial pressure for more than one or two minutes before the tracing was taken. Three or four such tracings at different arterial pressures, forming a series the members of which were comparable amongst themselves, were taken upon each drum.

Outside of the variations of arterial pressure the only condition which was liable to change during a series was the pulse-rate. Any change in pulse-rate would produce an alteration in the relations of systole and diastole, and destroy the value of the series. As a matter of fact many series were rejected on this account. Since, however, the tracings of any one series were always taken from the same flask of blood, the temperature and therefore the pulse-rate remained constant in the majority of cases. To obtain the duration of the systole and the diastole at each arterial pressure, vertical lines were drawn from the tuning-fork curve to the beginning and end of the heart-beat, for ten successive beats. The time of each systole and diastole was then counted out, the average taken, and the necessary correction made for the arc described by the pen.

Very soon after the commencement of our work a difficulty presented itself in determining at what point to reckon the be-

ginning of a systole. In some heart-beats, especially those in which there was a slow pulse-rate, the ascending limb of the curve was of the character shown in Fig. 3. The curve as seen in this figure, does not rise from the base line with uniform rapidity; there is at the beginning of the wave a slow rise, which later suddenly increases in steepness. It seemed to us that the preliminary rise was merely the indication of the auricular beat, and that the systole proper of the ventricle began at the commencement of the steep and sudden ascent of the wave. So long as the pulse-rate remains the same, as it does in each single series of observations, and the arterial pressure is not lowered below the limit at which the heart is well nourished, it really makes no difference whether the systole is counted from the bottom of the wave or from the beginning of the steep rise, as far as the effect of arterial pressure upon the time relations of the phases of the heart-beat is concerned, since the difference would only affect the absolute length of the systole and not its comparative relation to the length of the diastole at different arterial pressures. If we wish to make a comparison between the times of the systole with different pulse rates, then it becomes necessary to settle this point. We had it in mind to go on to the effect of changes of temperature on the time relations of systole and diastole, and therefore carried out several experiments for the purpose of determining which point of the curve indicates the actual beginning of the ventricular systole. The result at which we arrived is that the first shallow rise is really caused by the auricular contraction, and in counting out our tracings we always began to reckon the systole from the beginning of the steep rise. So that our figures indicate, for the given pulse rates, the absolute length of the systole and the diastole in the dog's heart.

The experiment which we made to determine this point was to take simultaneous tracings, in the way described, from both auricle and ventricle. A catheter was introduced into the right auricle through the superior vena cava, and into the left ventricle through a slit made in the auricular appendage; each catheter was connected with a Fick manometer. It was necessary to make the auricular catheter larger and to connect it with its manometer by means of wide lead tubing, in order to obtain distinct auricular waves. This had the disadvantage that distinct oscillations of the

large column of water took place, and were evident in the tracings, though this was of little consequence for the question in hand. The exact position of the catheters in the heart in these, as in all the other experiments, was determined by post-mortem examinations. It was not possible to make any series of observations at different arterial pressures with catheters in both auricle and ventricle. The heart was usually injured to such an extent by the operation that it soon became too weak to pump the blood to any considerable height, and shortly died. Several such experiments were made, however, in which the heart beat normally for some length of time. Figure 4 gives a portion of a tracing taken in this way. The two pens in this case were un-fortunately not writing in the same vertical line; the pen of the manometer connected with the auricle, giving the lower of the two tracings in the figure, was about a millimeter in advance of the other. In the lower curve, given by the auricular manometer, it is seen that both the auricular and ventricular contractions are recorded. By comparing it with the upper curve, which was given by the manometer connected with the left ventricle, it is very evident that the short preliminary rise in the contraction wave of the latter is synchronous with the auricular contraction as given in the former.

From this, and other simultaneous tracings in which the con-traction wave was of a different form, we were led to the conclu-sion that the proper systole of the ventricle begins at the steep rise, and in all our tracings, as we have said, we have reckoned it from this point. When the pulse-rate is rapid, and there is no appreciable pause after the diastolic expansion, the auricular wave does not appear in the ascending, systolic limb of the wave, but at the end of the previous diastolic descent; the systole in such cases was counted from the beginning of the wave.

At the result of many series of observations, most of which are given in the following table, we are able to state that varia-tions of arterial pressure, between 50 and 160 mms. of mercury, have no direct influence whatever upon the duration of the systole or the diastole of the heart beat in the dog.

When the blood pressure sinks so low that the proper nutrition of the heart is prevented, there is a diminution in pulse rate and a consequent change in the time relations of the systole and diastole. (See Exp. II, Obs. A$_4$.)

Professor Martin, in his work on the effect of arterial pressure on the pulse-rate, placed the limit to which arterial pressure could be lowered without affecting pulse-rate at about 20 mms. of mercury. In some of the hearts used in our experiments this effect was manifested when the arterial pressure fell to 30 mms. of mercury, as in the case cited. On the other hand, we have no doubt that the arterial pressure might be raised to considerably more than 160 mms. of mercury without affecting the time of systole or diastole. In one observation, indeed, the arterial pressure was increased to 180 mms. without causing any change; the series as a whole in this case was not of a uniform pulse rate, and hence is not given in the table. The numbers given, 50 to 160 mms. of mercury, can be fairly assumed as the limit of probable variations of arterial pressure in living dogs of the size used in the experiments

In the table given below the pulse rates for the different observations cannot be taken as absolutely correct. The pulse-rate on the kymograph tracing was always estimated, as nearly as possible, for the ten seconds during which the drum, upon which the ventricular tracings were taken, was revolving. But owing to the irregularity of the tracing of a mercury manometer, especially when the kymograph to which it is attached is going rather rapidly and shaking the manometer more or less, errors of half a beat or more may easily be made in counting out. Whenever the difference in pulse-rate was greater than one beat in ten seconds the series of observations was rejected.

A consideration of the table will show that only in rare instances do the times of the systoles or diastoles, in any one series of observations, differ amongst themselves by as much as .01 of a second. And of the cases in which a difference as great as this occurs, it sometimes points to an increased length of systole with increased arterial resistance, and sometimes the reverse, appearing to indicate that the difference was probably owing to errors of marking. In the method adopted by us it is not always possible to mark with absolute precision the beginning or end of the systole, and errors of .01 of a second might readily be made in this way. In some forms of waves no such difficulty occurred, and the time of the systole or of the diastole for the ten waves counted out remained practically identical. In other forms more serious

Number of Experiment.	Observation	Pulse Rate in 10 seconds.	Arterial Pressure in mm. of Hg.	Duration of Average Systole in sec.	Duration of Average Diastole in sec.
I.	A_1	31.5	140	.121	.189
	A_2	31.5	58	.121	.189
	B_1	29.5	96	.133	.193
	B_2	29.25	140	.121	.197
II.	A_1	34.5	100	.120	.170
	A_2	35.	152	.121	.166
	A_3	35.	60	.118	.164
	A_4	31.5	27	.092	.226
	B_1	33.	106	.127	.179
	B_2	33.	149	.137	.170
	B_3	32.	60	.138	.177
	B_4	32.5	105	.137	.174
III.	A_1	30.5	101	.160	.170
	A_2		150	.162	.170
	A_3		65	.159	.174
IV.	A_1	27.75	100	.112	.252
	A_2		65	.117	.242
	A_3	28.	137	.118	.239
	A_4	28.75	65	.115	.232
	B_1	27.	100	.113	.256
	B_2	27.	118	.119	.246
	B_3	26.5	61	.119	.261
V.	A_1	30.5	121	.156	.166
	A_2	30.5	66	.157	.160
	A_3	31.	145	.157	.163
	B_1	31.75	101	.146	.163
	B_2	31.5	124	.146	.164
	B_3	31.5	65	.147	.169
	C_1	30.25	99	.157	.170
	C_2	29.5	135	.149	.177
	C_3	29.5	65	.145	.194
VI.	A_1	23.3	102	.253	.182
	A_2	23.	136	.246	.183
	A_3	23.	63	.250	.184
VII. Catheter in Left Ventricle	A_1	24.	99	.133	.251
	A_2	23.25	134	.146	.248
	A_3	23.	57	.147	.244
VIII.	A_1	31.5	104	.126	.200
	A_2	30.5	64	.129	.193
	A_3	30.	152	.132	.196
	B_1	30.5	107	.132	.195
	B_2	30.	160	.127	.208
	B_3	30.25	68	.130	.203
	C_1	30.5	100	.126	.194
	C_2	30.75	62	.129	.196
	C_3	30.5	151	.130	.198
	C_4	30.	63	.129	.205

differences were met.   In these cases we relied upon the average
to correct errors in counting, since a mistake was as liable to be
made in one direction as the other.   The great majority of ob-
servations given in the table show differences amongst the times
of the systoles or diastoles of less than .01 of a second.   In ex-
periment VIII, in which the pulse rate remained constant
throughout, the observations of each series are comparable not
only amongst themselves, but also with the observations of the
other series.

The general form of the wave of ventricular contraction is
shown in Fig. 1, which represents a portion of the tracings of
Series C, Experiment VIII.   The figure gives the actual size of
the tracing.

The form of the curve is quite different from that given by
Baxt for the dog, but very similar to that given by Hoffa and
Ludwig for the same animal.   Baxt's curve possesses a very
square top.   We never got any such appearance from our hearts
except in one experiment, in which, at the high arterial pressure
used, the heart had apparently about all the resistance opposed to
it which it could overcome ; an example of the curve obtained in
this instance is shown in Fig. 2.   As a general rule the wave of
contraction was more or less rounded at the apex, passing with-
out any abrupt break from the systolic ascent to the diastolic
descent, even at the highest arterial pressures.   That the apex of
the curve does not show any noticeable continuation in contrac-
tion, may possibly be owing to the rapid pulse-rate which fol-
lows after the vagi have been cut, although with the slowest
pulse-rate obtained, about 130 beats per minute, the character
of the curve in this respect was not altered.   Any such curve as
that given by Baxt seems to us to indicate that the last part of the
systole and the first part of the diastole have not been registered.
This makes but little difference if we define the duration of the
systole, as Baxt has done, as the period extending from the rise
of the wave until it again reaches the base line, and limit the
diastole to that period known as the heart pause, during which
the ventricular muscles are completely relaxed.   According to
this nomenclature the diastole will disappear altogether when the
pulse-rate is very rapid.   Such a limitation of the systole is, we
think, altogether wrong.   The systole of any portion of the heart,

as Moens has defined it, is that condition of the heart in which its muscle fibres are in action, and the diastole, therefore, properly begins at the commencement of the relaxation of the muscle fibres.

When the arterial pressure is lowered to such an extent that the nutrition of the heart probably becomes deficient, a slowing of the pulse-rate, as has been said, is the result. Together with this slowing of the pulse the systole of the heart is diminished in intensity and shortened in duration, while the diastole becomes lengthened. In one of our experiments, for instance, when the arterial pressure was lowered from 144 mms. to 28 mms. of mercury, there was a slowing of the pulse-rate from 29.25 beats in ten seconds to about 24 beats in ten seconds, while the duration of the systole fell from .134 to .095 of a second, and the diastole was increased from .207 to .301 of a second. With a heart well nourished our experiments, as far as they go, appear to indicate that as the pulse-rate becomes slower the time of both systole and diastole is increased, though not in the same proportion. We have not sufficient data, however, to make any positive statement upon this point. The relation of the time of the systole to that of the whole beat varies for different pulse-rates. For an average pulse-rate of 180 per minute, the average duration of the systole, according to our experiments, is about 40 per cent. or two-fifths of the whole time of the beat.

EXPLANATION OF PLATE XXXVII.

The figures give the actual size of the tracings obtained.

FIG. 1. A portion of Series C, Experiment VIII, showing tracings at different arterial pressures. The correction for the arc described by the pen, .015 of a second for C_1, C_2, and C_4, and .02 of a second for C_3, is to be added to the systoles and subtracted from the diastoles.

FIG. 2. Shows the square-topped waves obtained in one of the experiments when a high arterial pressure was used.

FIG. 3. Showing the auricular contraction at the beginning of the ventricular wave.

FIG. 4. Simultaneous tracings from the right auricle and left ventricle. The lower curve is from the manometer connected with the auricle and is to be moved to the left a distance of 1 mm. The con-

traction of the right ventricle as well as of the right auricle is registered by the manometer. The upper curve is from the manometer connected with the left ventricle. The tuning-fork curve marks hundredths of a second.

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ERRORS IN PLATE.

Fig. 1. C_4, 4th systole from the left, instead of .10 of a second should be .11 of a second.

C_2, 1st and 2d systoles from the left, instead of .10 of a second should be .115 of a second.

5th systole from the left, instead of .10 of a second should be .11 of a second.

C_1, 2d systole from the left, instead of .10 of a second should be .11 of a second.

The plate was drawn from the original tracings, but two attempts of the printer have failed to get the chronographic tracings correctly transcribed.

NOTES ON THE MEDUSAE OF BEAUFORT, N. C.

Part II.[1] By W. K. BROOKS, Associate Professor of Biology, Johns Hopkins University.

Turritopsis Nutricula (McCrady).

Oceania nutricula (McCrady). *Modeeria multitentacula* (Fewkes). *Modeeria nutricula* (Fewkes). *Turritopsis nutricola* (Haeckel).

This is one of the most abundant medusae at Beaufort during the summer months, and I have been able to verify the extreme accuracy of McCrady's graphic account of the structure and habits of the adult. The larva is probably a deep-water form, as it was found only once, notwithstanding the abundance of the medusa.

The Larva.—The only colony of the hydra which I obtained was scraped from the piles of the steamboat wharf at Morehead City, seven or eight feet below low-tide mark. It lived for two weeks in the house, and set free great numbers of hardy medusae, which were reared without difficulty. The upright stems, from one-third of an inch to half an inch high, bore large terminal hydranths, as well as smaller ones scattered irregularly along the stem on short stalks. The long fusiform body of the hydranth carries from eighteen to twenty thick, short filiform tentacles, which are arranged in three or more indefinite whorls. The medusa buds grow around the stem just below the hydranth, and are carried on short stems. The perisarc is not annulated, and it forms a loose cylindrical sheath around the main stem and the short branches which carry the lateral hydranths and the young medusae, while the latter are closely invested by a thin capsule of perisarc. The sheath on the stems is thick and crusted with foreign matter. It terminates abruptly by a sharp collar just below each hydranth. The young hydranths and medusae are budded off at this point, but soon become entirely sheathed in

[1] For part I, see this Journal, Vol. II, p. 135.

perisarc by the growth of the stem. The pale yellowish-red
hydranths are very similar to those of *Tubiclava* (Allman.)

Metamorphosis of the Medusa.—The little medusa remains
attached to the stem for some time after the rupture of the
sheath of perisarc. At this time it is nearly spherical, and
covered with large conspicuous ectoderm cells. Its eight short
tentacles are thrown backwards in contact with the outer surface
of the bell, and their tips are hooked or bent upon themselves.
This position of the tentacle renders the bulb at the base, with its
ocellus, very prominent.

The medusa, when set free, has eight tentacles, a thin globular
bell, and a short simple proboscis. When swimming the tentacles
are bent into hooks and thrown back against the umbrella, which
is lengthened and emarginated during each contraction. When
at rest the height of the umbrella is about equal to its diameter,
and it forms a spherical segment almost equal to a sphere. The
tentacles are capable of extension to a length equal to about
twice the diameter of the umbrella, and when the animal is at
rest they are stretched out almost horizontally, and the distal
half is bent downward a little, forming an obtuse angle near the
middle of the tentacle. The interradial tentacles lie nearly in
the plane of the bell-margin, and the perradial tentacles a little
lower. The tips of the extended tentacles are slightly clavate,
with a spot of dark orange pigment. The length of the pro-
boscis is about two-thirds the height of the umbrella, and its
upper and lower ends are smaller than the middle. The
mouth is simple, and the endoderm of the oral end of the pro-
boscis is very thin, but just below the constriction at the aboral
end it becomes very thick ; the thickened area arching outwards
on to the sub-umbrellar surfaces of the radiating tubes.

This thickening of the endoderm cells of the aboral end of the
stomach is characteristic of Turritopsis; and in a specimen a
week old, the whole upper half of the proboscis is filled by four
great masses of very large endoderm cells, which meet in the
central axis and run out for some distance into the radiating
tubes. The singular structure which is thus formed has been
described by various authors as a peduncle, but it is not at all
the same as the gelatinous projection from the substance of the
umbrella which, in many medusae, hangs down into the stomach.

As the medusa grows the proximal ends of the radiating tubes are drawn down into the cavity of the umbrella, until, in specimens two weeks old, the stomach is suspended some distance below the sub-umbrella, by a transparent mass of large cells, meeting in the central axis and perforated by the four tubes. In the adult this body almost entirely fills the upper half of the umbrella-cavity. In a medusa a week old the oral lobes have appeared, and are fringed by the large projecting lasso-cells which have been noticed by McCrady and others. At about this time the reproductive organs make their appearance on the proboscis at the lower ends of the masses of endoderm cells. The tentacles are still only eight, and no more were developed in the medusae which I reared from the larva, but I captured many specimens in the same stage and at all the following stages up to maturity.

In specimens from a week to two weeks old the lower surface of the very wide velum is pushed out to form eight hemispherical pouches ; four of them perradial and four interradial, in the planes of the eight tentacles. These pouches project so that they are visible in a profile view below the free edge of the umbrella.

CUNINA OCTONARIA (McCrady).

McCrady's remarkable discovery that the young of this species exists as a parasite within the bell of Turritopsis, a medusa belonging to a totally different group, is of so much interest that I was well pleased to have an opportunity to verify it at Beaufort during August and September, 1882. Since McCrady's paper was published no one has succeeded in rediscovering these larvae, and as both Cunina and Turritopsis occur at Beaufort, the latter in considerable numbers, I had kept a sharp watch for them for nearly three years before I found them. Near the end of July, 1882, I found a single specimen of Turritopsis filled with the larvae, and from this time until the end of the season they could be obtained in great abundance. I was therefore able to verify McCrady's accurate account of the metamorphosis, and to add a number of new points which I hope to publish soon in an illustrated paper.

NEMOPSIS BACHEI (L. Agassiz).

Nemopsis Gibbesii (McCrady).

This medusæ is quite common at Beaufort during the spring and early summer months, and specimens were found at all stages of growth. There does not seem to be any reason to doubt its identity with the northern form, and Agassiz' specific name must therefore be retained in place of McCrady's name.

The Larva.—Most writers upon the subject have questioned the relationship between the floating hydroid found and described by McCrady, and Nemopsis, and my observations show that the medusa is derived from a fixed hydroid closely related to *Bouganvilleia* and *Endendrium*.

On May 29th, 1882, the dredge brought up from about twelve feet of water in Newport river, a piece of decayed wood covered with a small Endendrium-like hydroid about an inch high. Each main stem gave rise to three or four short alternating branches, and these, as well as the main stem, ended in hydranths, which were sharply separated from the stem by a fold or collar. The thin transparent horny ectosarc extended almost but not quite up to this fold, and there were two or three irregular annulations on each side branch close to the main stem. The hydranth carries twenty-four long slender tentacles, with their proximal ends in a single circlet, but with their tips bent alternately backwards and forwards, thus forming two circlets. The very extensible funnel-shaped proboscis is sharply distinguished from the body of the hydranth, and the hydra therefore resembles *Endendrium*, as described by Allman, more than it does *Bouganvilleia* in this particular.

The six or eight medusa buds are arranged in a ring around the body of the hydranth, about midway between the bases of the tentacles and the proximal end of the body. The various medusæ in this ring are in different stages of growth, and only one is usually set free at a time. The terminal hydranths and those near the end of the main stem have no medusa buds, as these seem to be developed only upon the older hydranths.

The Metamorphosis of the Medusa.—The medusa is very small when set free, and it is flattened and folded together so that the proboscis projects out of the umbrella. In half an hour or an hour it expands and begins to swim. It is then about two

one-hundredths of an inch high, and the diameter is a little less than the height. The proboscis is short and simple, without oral tentacles, and the umbrella is about as thick at its sides as it is in the oral axis. Most of the specimens had four perradial tentacles—one at the end of each radiating tube. In others there were six tentacles, arranged in this way, and in these

```
              3. 2
               |
  1.———————————|———————————1.
               |
              2. 3
```
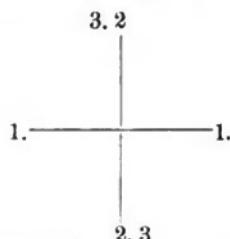
the tentacles 1. 1. were much larger than those marked 2. 2., and these again larger than 3. 3.—the latter being very small transparent buds in most specimens. The order of appearance of the tentacles varies considerably. In one medusa, twenty-five one-thousandths of an inch in diameter, they were like this—

```
             3. 2. 4.
               |
  1————————————|————————————1
               |
             4. 2. 3
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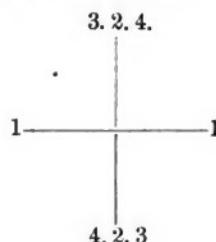
No. 1 being larger than No. 2, this again larger than No. 3, while No. 4 was a very small bud.

In another specimen of the same size they had the arrangement shown in this diagram—No. 1 being in each case larger

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              1. 2.
               |
  2.———————————|         1.
  1.———————————|         2.
               |
              2 | 1.
```
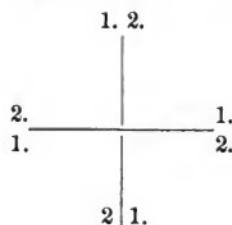
than No. 2, and more directly in the line of the radiating tube. In this specimen, twelve days later, after it had grown to a diameter of four one-hundredths of an inch, the arrangement was like this, with an ocellus in 1 and 2. At this stage all four ten-

```
              4. 2. 1. 3.
                   |
    3.             |             4
    1. _____|_____ 2.
    2.             |             1.
    4.             |             3.
                   |
              3. 1. 2. 4
```
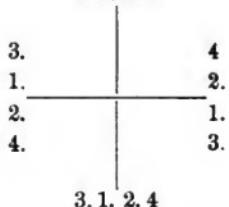
tacles in each cluster were of about the same size; but in a younger specimen, which was taken with the tow-net on May 9th, and which was three one-hundredths of an inch in diameter, No. 4 in each bunch was a small transparent bud. The oral tentacles appear when the medusa is about three one-hundredths of an inch in diameter. They are simple at first, but they soon become forked at their tips, and each of these forks becomes forked in the same way, and so on. It is hardly possible to give a clear account of the changes in the shape and outline of the umbrella without figures, but I am sure that, when my figures are published, they will prove the specific identity of the northern with the southern form.

PHORTIS GIBBOSA (McCrady).

Eirene gibbosa (L. Agassiz). *Irene gibbosa* (Haeckel).

As all the other species of Irene have marginal cirri, the absence of these structures in this form seems to justify the retention of McCrady's generic name. It is a very rare medusa, and McCrady gives no figure of it, although I have in my possession a sketch made by him from memory. The occurrence of the medusa has never been noted by any one except McCrady. Specimens were occasionally met with at Beaufort during the summer months, and I had therefore been able to secure a pretty complete series of the older stages, when, in September, 1882, I obtained the hydra stage in great abundance, and reared from it hundreds of young medusae.

The Larva.—On September 19th, 1882, quantities of stems of Aglaophenia were torn up by a gale and thrown upon the beach at Fort Macon. Attached to these stems were specimens of a peculiar campanularian hydroid. A long slender hydrorhiza runs along the stem of Aglaophenia, and gives rise, at pretty regular intervals, to short annulated branches, some of which terminate in hydranths and others in reproductive calicles, which do not differ very greatly from the hydrothecae either in size or in shape. The hydrothecae are trumpet-shaped, slightly curved, and they taper gradually from the base, which is no larger than the short stem, to the wide, flaring, reflected opening. The hydranth has a long slender body and about twelve tentacles, with rings of lasso-cells.

The gonotheca is very similar to the hydrotheca in size and shape, as well as in its position upon the stem. The chief differences are that the gonotheca tapers somewhat more gradually towards the stem than the hydrotheca, the annulations run up it for half its length or more, and its distal end is less flaring. The blastostyle runs along one side of it, to terminate in a club-shaped tip or manubrium, and medusa buds are placed along one side of it. There are only three or four of these, increasing in size from the base to the free end.

The Metamorphosis of the Medusa.—Only one medusa escapes at a time—the largest one nearest the distal end of the blastostyle—and, as soon as it is set free, it expands or unfolds so as to become about as long as the entire gonophore.

As soon as the hydroids were captured each specimen was placed, alone, in a tumbler of sea-water, and, when the laboratory was reached, each tumbler was found to contain hundreds of swimming medusae. These were carefully picked out with a dipping tube, and the hydroids were then placed in larger aquaria, where they lived for nine days and continued to throw off medusae, although the number set free daily was very much less than the number set free within a few minutes after the specimens were found. The rarity of the adult medusa stands in marked contrast with the hardy and prolific nature of the larva, and as the latter were found on this occasion in great abundance, I conclude that its proper home must be at some distance from the shore, and that the stems of Aglaophenia upon which they were found had been torn up from deep water.

When set free the ex-umbrella of the medusa is regularly curved, and its height is a little greater than its diameter. The sub-umbrella, on the other hand, is bent upon itself about half way up, at an obtuse angle, and the lower or free half of the umbrella is about twice as thick as the upper half. The proboscis is very short and is divided into two portions—an upper flat division which runs out along the radiating tubes for about one-sixth of their length, and a pendant portion of about one-fifth the height of the umbrella, and folded into four oral lobes. There are no traces of marginal sense organs at this stage, and the arrangement of the tentacles, in all my specimens, was somewhat peculiar, as shown in the diagram.

There are four perradial tentacles, of which one (3) is very much smaller than the others, and consists, in some specimens, of a bulb only, the lash being undeveloped. Opposite this is a somewhat larger tentacle (2), with a short lash. The two remaining perradial tentacles (1 1) are fully developed and alike. Their lashes are very slender and delicate, and may be thrown out to two or three times the diameter of the bulb. Between these four perradial tentacles four interradial tentacles (4 4 4 4) are represented by bulbs without lashes. In one of the quadrants there are two small protuberances from the wall of the circular tube—the adradial tentacles (5 5). They are placed midway between the perradial and interradial tentacles, and there are no traces of them in the other three quadrants. At this time there are no otocysts, and lateral cirri are totally absent at this stage, as they are at all later stages.
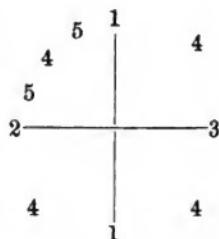
At the end of the first eighteen hours the shape of the medusa has changed completely. When contracted in swimming, its height is nearly equal to its diameter; but when it is at rest the diameter is about twice the height, so that it is no longer globular but saucer-shaped. The upper half of the umbrella has begun to thicken to form the peduncle, and it is now about as thick as the lower half, except at the angle in the sub-umbrella, where it is still thin. The four perradial tentacles are all furnished with lashes, but these are still absent in the interradial

tentacles, although these have grown larger. There are now two adradial thickenings of the circular tube in each quadrant.

In a specimen one-fourth of an inch in diameter there are sixteen fully-developed tentacles and sixteen thickenings of the circular tube, without lashes. The four perradial tentacles and the four interradials are equal and similar, while the eight primary adradials are somewhat smaller. There is an otocyst with one otolith on each side of the base of each tentacle, making thirty-two in all.

In a specimen two-thirds of an inch in diameter there are forty tentacles, and sixty in one an inch wide.

AMPHINEMA APICATUM (Haeckel).

Saphenia apicata (McCrady). *Stomotoca apicata* (L. Agassiz). *Stomatoca apicata* (Fewkes).

This medusa is not very common at Beaufort, although specimens are found occasionally all through the summer. The structure of the adult has been well described by McCrady and Fewkes, and I have little to add to their accounts.

The Larva.—The hydra stage was found on three occasions at Beaufort between July 5th and July 11th, 1882, on the lower surface of the shell of the living *Limulus*, fastened to the sandtubes of *Sabellaria*. It is a *Perigonomus* very much like *P. minutus* (Allman). The simple unbranched slender upright stems are from eight one-hundredths to one-tenth of an inch high, and their bases are encased in the sand-tubes of the annelid so that the presence of a creeping stolon could not be observed. The flexible stem is covered for one-half or two-thirds its length by a delicate, closely adherent film of perisarc, to which foreign particles are attached. The stomach occupies about one-fourth or one-fifth the total length of the stem, from which it is separated by a slight constriction. There were ten tentacles in each of the thirty or forty specimens which I examined, and, when fully extended, they point alternately backwards and forwards—those pointing forwards being a little longer than the others. The medusae are attached by very short peduncles along the stems, but as most of them were set free before the specimens could be examined, the mode of attachment could not be carefully studied.

Each colony of larvae was placed by itself in a bottle of sea-water as soon as it was found, and, when the laboratory was reached, each bottle was found to contain hundreds of minute but very active medusae. They proved to be quite hardy and lived for more than a week in aquaria, although the great length and delicacy of the tentacles caused great difficulty in rearing them, as the tentacles became entangled with each other and with the sides of the jar, so that the medusae could not be drawn into a dipping tube without injury, and many were destroyed each time the water was renewed.

Metamorphosis of the Medusa.—When the medusa is set free there is no trace of the apical process, which is not a larval structure, but an adult characteristic. The bulb is about twice as high as wide—the height being about twenty-five thousandths and the diameter about thirteen thousandths of an inch. The wall of the umbrella is thin, and its surfaces are nearly concentric and regularly curved. The proboscis hangs down to about one-half the height of the umbrella cavity, and ends in a circular mouth. The stomach is a little enlarged at its base, where it joins the radiating tubes. There are two tentacles with large bulbs, faintly tinged with pale orange. The long delicate lash springs abruptly from the bulb, and its base is very little larger than its tip. Immediately after the medusa is liberated the length of the tentacle is four or five times the height of the umbrella. Alternating with the two opposite tentacles there are two small pigmented perradial bulbs without lashes.

In a medusa three days old and thirty-five one-thousandths of an inch high, the apical process is present as a short, solid, rounded projection from the aboral pole. The tentacles are from ten to twenty times as long as the height of the bulb, and four pigmented interradial enlargements of the wall of the circular tube have appeared midway between the four perradial bulbs. The length of the proboscis is now a little more than half the height of the sub-umbrella.

When five days old the medusa begins to assume the adult form. The apical process grows rapidly, and becomes pointed or conical, the lower or free half of the umbrella becomes thicker than the upper half upon which it is bent at an angle. The four oral folds have appeared, and the upper end of the proboscis is

slightly enlarged, probably by the growth of the sexual elements. The tentacle tapers more gradually at the bulb, and the lashes and marginal enlargements are relatively a little larger than they were at an earlier stage.

In specimens eight days old the process is equal to or greater than half the height of the umbrella, and the medusa has essentially the adult form, except that the marginal enlargements are much larger relatively and less numerous than they are in the adult. I was not able to keep them longer, as the tentacles, fifteen or twenty times as long as the height of the umbrella, became entangled with each other and attached to the sides of the glass jar, so that I was not able to remove the animals to change the water without injuring them.

Liriope Scutigera (McCrady).

This is one of the most abundant medusae at Beaufort, and there is no difficulty in obtaining a supply of segmenting eggs and young medusae. The eggs are very small and transparent, and, as they develop with great rapidity, they are very favorable subjects for embryological work. My results agree perfectly with those of Metschinchoff, and there is no difficulty in witnessing the actual delamination of the inner ends of the cells of the developing egg.

THE ACTION OF ETHYL ALCOHOL UPON THE DOG'S HEART.

By H. NEWELL MARTIN, M. A., M. D., D. Sc., Professor in the Johns Hopkins University, and LEWIS T. STEVENS, B. A., Fellow of the same.

The physiological action of alcohol is a subject in connection with which very much has been written. In the Index Catalogue of the Library of the Surgeon-General's office there are more than one hundred and fifty separate references under the title " Alcohol, physiological effects of." From this vast mass of literature bearing on a subject which has been so often prominent in social and political discussions, very much may, of course, be at once eliminated as of no immediate interest to the physiologist or therapeutist in his capacity as such. It contains no original experiments, and is mainly a rhetorical and uncritical account of the work of others, often also described with a mental bias. After throwing aside these productions of the orators and essayists, there still remain numerous articles professing to deal with the physiological action of alcohol which can hardly be accepted as so doing, for in many cases all sorts of alcohol-containing drinks have been administered to men or the lower animals, and the results, if any, set down as due to the alcohol only. That this is not justifiable a moment's consideration will make clear, for it is well known that in different wines and spirits various substances are present which have potent action on the system, and cause these drinks, quite apart from the percentage of alcohol in them, to produce each its own characteristic effect, not only immediately after consumption, but, when taken in excess, remotely and permanently ; as illustrated by the different pathological states to which they give rise or predispose. It is to this cause undoubtedly that the very discordant statements of various workers are mainly due; while there has also been a good deal of careless experimenting, such as the injection of large doses of 90 per cent. alcohol into the alimentary canal and the ascription of the consequences to absorbed alcohol, quite regardless of the intense local irritation which must have been set up in the stomach or rectum of the animal experimented upon. During

the last thirty years more careful work with reasonable doses and dilution, and with attention to the kind of alcoholic liquid used, has given better results. So far at least as the pulse is concerned, it seems fairly settled that alcohol diluted with water and in doses sufficient to produce transient disturbance of the mental faculties, has no effect on the pulse-rate of healthy men or other mammals, though even here there is not absolute agreement. Zimmerberg,[1] whose paper is the most satisfactory of all those on this subject with which we are acquainted, found no pulse alteration caused by alcohol in dogs and cats when the animals were not tied down. Rabbits, on the contrary, showed a quickened pulse, but this seemed due to scare, for the same phenomenon was observed when a little water was injected into the animal's stomach. He also could discover no pulse quickening in man. Dr. Edward Smith,[2] however, found his own pulse quickened by alcohol, while that of Mr. Moul was unaffected. As Dr. Smith makes no statement as to whether he was accustomed to the daily use of alcohol, it seemed possible that he was an habitual abstainer, and that the pulse-quickening action of the alcohol in his case depended upon the fact that his system was quite unaccustomed to it. As this point seemed of interest and perhaps of practical importance, we asked a friend, aged about twenty-six, and who had never, so far as he knew, drank anything containing alcohol, to allow us to make an observation upon him. He kindly consented, and we give here the result before proceeding to the main series of our experiments. The alcohol used in this case and throughout our researches was that prepared by Squibb, and sold as " Absolute Alcohol " of sp. gr. 0.7850 at 25° C., and warranted to contain not less than 99.75 per cent. of pure ethyl alcohol. Mr. J.'s last meal was taken at 7 P. M. At 9 P. M. he lay down on a bed, and his pulse-rate was noted at intervals for an hour. At 9h. 05m. it was 74 per minute, and varied between that and 71.5 until 9h. 30m.; he then became drowsy, and this and the recumbent posture brought the pulse down to 68 at 9h. 58m. At 10h. 08m. he was roused; at 10h. 10m. told he was to be given the alcohol. The substance really administered was, however, only some sugar and water—the object being to see what effect, if any, the idea of taking the drug (which might well excite a person accustomed to regard it somewhat in the light of a poison)

would have on the pulse. There was a transient quickening to 73 per minute, but this was probably merely due to rising from the recumbent position in order to drink. At 10h. 31m. P. M., when the pulse had fallen to 70, 15 cub. cent. of alcohol in 50 cub. cent. of water were given. This caused no rise of the rate of heart-beat greater than two beats in a minute, and this only lasting a few minutes, and easily accounted for by the muscular effort involved in changing the posture. At 10h. 52m. the pulse was again 70 per minute, and thenceforth until the final counting, at 12h. 10m. A. M., its rate lay between 72 and 67 per minute—on the whole slowing towards the close of the experiment. This slowing can hardly have had any dependence on the alcohol, as it is well known that the pulse normally becomes less frequent towards midnight, and especially in a person who has lain for hours at rest. That the dose of alcohol was sufficiently large was evidenced by the dizziness produced by it.

We here give in tabular form the results of the experiment just described.

Hour.	Pulse-rate per minute.	Notes.
P. M.		
9h. 05m.	74	Subject lay down on bed at 9 P. M.
15	75	
25	71.5	
27	73	
30	72.5	
42	67.5	Drowsy.
50	69	"
58	68	"
10h. 08m.	—	Aroused.
10	73	45 cc. of water with sugar in solution administered immediately before.
15	72	
25	71	
30	70	
31	—	15 cc. alcohol in 50 cc. of water given.
35	71	
40	70	
45	72	Complains of slight dizziness.
52	70	
11h. 00m.	67	
07	69	
19	68	
25	67	
35	68	
48	70	
12h. 00m. A. M.	68	
12h. 10m.	69	

Combining this experiment on a teetotaller with those of previous workers, we think it tolerably certain that moderate quantities of pure ethyl alcohol so diluted with water as to have no local irritant action, exert no influence on the pulse-rate of healthy men. Possibly the contrary result obtained by Dr. Edward Smith is to be explained by the fact that he was experimenting upon himself. Although practised in so doing, he may not have always been able to suppress such an amount of interest in the result as amounted to a nervous excitement sufficient to influence his pulse. It is, perhaps, necessary here to definitely state that the above conclusion applies only to ethyl alcohol, and not to various wines and spirits. As regards several of these, the evidence collected by Dr. Edward Smith and others points the other way Some quicken the pulse, and, so far as diseased persons are concerned, the clinical evidence seems conclusive that, under certain conditions, some alcoholic liquids will remarkably diminish the rate of heart-beat. In the treatment of the sick, however, pure diluted ethyl alcohol has rarely been used, and it may be that the influence observed on the pulse-rate is a specific action of some of the other constituents of the liquids administered.

When a substance acts upon so many different systems of the body as alcohol does, it becomes no easy matter to get at its immediate specific action upon any one organ ; yet a knowledge of this may be of primary importance. A given substance, for example, is known to raise arterial pressure ; perhaps it is often a matter of no consequence whether it does this by increasing the heart's work or by constricting the arterioles ; yet obviously circumstances may arise, *e. g.* a greatly weakened heart, when the administration of a drug constricting the arteries would perhaps temporarily increase arterial pressure, but in so doing throw so much extra work on the feeble heart as to lead to disastrous results. To raise therapeutics from empiricism or guesswork it is essential to know precisely the action of each drug on each organ in the body, and then its action upon them when working together in the living man. By the combination of careful observations at the bedside, with experiments made in physiological laboratories on the action of substances on healthy animals, and in laboratories of experimental therapeutics on healthy and diseased, we

may hope in time to know, at least with tolerable exactness (for there will always be individual idiosyncrasies to be met and combated) exactly what any dose given to any patient is going to effect in him. The educated physician does not now prescribe as his predecessor would have done, a dose of salts for every case of constipation ; he selects his purgative to suit the particular case and in accordance with his diagnosis of the seat of the trouble and his knowledge of the physiology of the alimentary organs and the specific action of the drug. To clearly establish for every substance used in medicine, first its special action upon each organ when isolated, and then its action upon each organ when that organ is in vital connection with all the rest, is a task of almost appalling magnitude ; but in proportion as it is accomplished will medicine become a trustworthy art based on scientific knowledge. Fortunately so much has been done of late years, especially in physiological and pharmacological laboratories, as to show that the task is not hopeless.

The investigation whose results are given in the following pages was undertaken with the hope of contributing some little to the attainment of the end above described, and also with the view of testing the availability of the dog's heart, isolated from all other organs of the body except the lungs, for therapeutical research. The latter subject seemed well worth investigating, as the hearts of frogs and reptiles, which have hitherto alone been experimented upon as regards the direct action of drugs upon the organ, differ in many fundamental points of anatomy, physiology, and nervous supply from the heart of man, while the dog's heart is practically identical with it in structure and working.

The animal having been narcotised by a large dose of acetate of morphia subcutaneously injected, or by the inhalation of the vapor of a mixture of ether and chloroform, the heart was isolated essentially in the manner described in a previous number of this journal (Vol. II, p. 213, plate XV). Certain modifications in the method, however, require mention.* Instead of allowing the right carotid to pump out through the tube q (Plate XV), and regulating the pressure in the aortic arch by opening

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* The modifications here described are so inconsiderable and easily intelligible that it has not seemed to us necessary to illustrate them by a new plate.

the stop-cock 22 more or less freely, the cannula inserted into the artery was attached to a long rubber tube which was led through the top of the warm chamber, in which the heart lay, to a height of several feet, where it ended in an outflow orifice. By varying the height of the point of outflow any desired arterial pressure could be easily obtained. We usually chose such a height as gave a mean pressure of 100 to 140 mm. of mercury, measured by a manometer connected with the left carotid, which recorded upon the paper of a kymograph, and thus also enabled us to count the pulse. We may at once dismiss the latter by saying that the doses of alcohol given by us had no effect upon its rate, thus confirming the results of the majority of recent observers.

In some cases the method was modified by tying up the right carotid instead of the aorta, and inserting into the latter a cannula of thin brass, as large as it would admit. This cannula was pushed up to the origin of the left subclavian and firmly tied there. To its distal end was connected a wide rubber tube, which led through the top of the warm chamber and ended in an outflow tube which could be raised or lowered at will. This modification was adopted to secure to the left ventricle a wide outflow channel, and thus eliminate a possible source of error due to its having only one carotid through which to empty itself. As will be seen subsequently the result was the same whether the left ventricle had only the carotid through which to force its contents, or a tube of the full diameter of the thoracic aorta. This might perhaps have been expected, as the height to which the column of blood had to be pumped was, in both cases, arranged with reference to the diameter of the tube through which it was forced, so as to give about the same pressure in the aortic arch; in other words, to oppose the same resistance to the systole of the left ventricle.

The nutrient liquid sent to the heart was supplied from four Mariotte's bottles, either of which could at will be connected with the organ. One of these flasks, at the commencement of the experiment, contained two litres of fresh defibrinated dog's blood, mixed with 500 cub. cent. of 0.75 per cent. solution of sodium chloride in distilled water. At the commencement of an experiment this flask was put in connection with the superior vena cava, and supplied the right auricle under a pressure equal

to that of a column of the blood mixture fifteen centimetres in height. This supply-pressure was the same for all the four flasks, as they stood on the same level, and, as repeated trials showed, gave rise, when the cannula usually inserted into the superior cava was disconnected from that vessel and allowed to pour into a beaker, to a greater flow of blood than the left ventricle ever pumped out in an equal time; so that the heart always had opportunity to take up more blood than it accepted.

The blood received by the right auricle from the first Mariotte's bottle having passed through the lungs, was finally sent from the left ventricle through the outflow tube connected either with the right carotid or with the aorta. From the outflow tube it poured into a funnel from which it passed back into bottle No. 2, where it collected; this bottle being meanwhile in free communication with the atmosphere, but shut off from the heart. When No. 1 was nearly empty and No. 2 full, by turning a couple of stop-cocks, No. 2 was cut off from direct connection with the outer air and converted into a Mariotte's flask, and at the same time placed in communication with the superior cava. No. 1 was, simultaneously, cut off from connection with the heart and arranged to receive the blood pumped out by the left ventricle and now supplied to the heart by No. 2.

One of us stood by the kymograph and looked after it; the other stood by the outflow tube. The former at intervals of a few minutes gave the word "get ready," and a few seconds afterwards "go." The other then immediately turned the outflow tube connected with the left ventricle so that it emptied into a beaker held in his hand. At the expiry of fifty-five seconds from the word "go" the warning "get ready" was again given, and at the end of a minute, upon a second utterance of the word "go," the collection in the beaker was stopped. The blood collected during this minute was measured and noted; and soon afterwards a new measurement of the quantity pumped out by the heart in a minute made in like manner. When bottle No. 2 was nearly empty and No. 1 full, the stop-cocks were reversed and the heart fed from No. 1; and so on as often as necessary. The blood collected for measurement was poured back through the funnel into the bottle which happened to be the receiving one at the moment. When such measurements made five or six consecutive times

agreed within a few cubic centimetres, the heart was considered fit for the examination of the action on it of alcohol-containing blood.   Bottle No. 3 contained when the experiment commenced two litres of defibrinated dog's blood.   As soon as it was ascertained that the heart was working with fair uniformity, 500 cub. cent. of 0.75 per cent. warmed sodium chloride solution to which alcohol had been added were mixed with the contents of No. 3. The quantity of alcohol used was such as to form either 0.25 or 0.5 per cent. of the whole; or, put in another way, 25 or 50 parts in 10,000.   The total quantity of alcohol administered did not exceed in any case which we here record (larger quantities were given in other experiments with marked pathological results) 10 cubic centimetres, an amount contained in about ⅜ oz. of good brandy.   It must, however, be borne in mind that under the conditions of our experiments the only organs concerned were the lungs and heart, and that when alcohol is swallowed much of it may be held back in the liver or eliminated by the kidneys.   It is therefore probable that much larger quantities of alcohol than those we employed might be administered by the mouth and absorbed and removed from the whole body without producing that influence upon the heart which our experiments demonstrate.   When the alcohol-containing Mariotte's bottle was connected with the heart, the stop-cocks were so turned that the blood pumped out flowed into bottle No. 4; and while the heart was fed from No. 3, measurements of the blood pumped out in a minute were made in the manner above described.   After the action of the alcohol had fully manifested itself, a bottle (No. 1 or 2) containing no alcohol was connected with the heart; if no marked recovery took place the experiment was rejected, as the diminished work might have been due to gradual death of the isolated heart, independent of any specific action upon it of the alcohol.   When unmistakable recovery took place the experiment was recorded as a satisfactory one, even though the heart did not regain completely its original working power.

Care was of course taken to keep the blood supplied to the heart of as uniform a temperature as possible.   Its temperature was observed by means of a thermometer inserted into the supply tube close to its attachment to the superior vena cava.

In a preliminary and general way our results may be stated as

follows: *When defibrinated blood containing ½ of one per cent.
by volume of ethyl alcohol is supplied to an isolated dog's heart
which has been hitherto working with uniformity, the invariable
result is a very rapid and marked diminution in the work done
(indicated by the quantity of the blood pumped out from the
left ventricle) by the heart in a given time. When the blood
contains only ¼ of one per cent. of alcohol the result is, in most
cases, the same, but sometimes is little or none. After the action
of the alcohol has been fully manifested the heart can in many
cases be restored to its original working state if supplied with
defibrinated blood containing no alcohol.* Blood containing but
one-eighth of one per cent. of alcohol exerts no influence upon
the work done by the heart, at least for several minutes.

As the heart was, under the conditions of the experiment,
isolated from all extrinsic nervous control, and supplied under
exactly the same pressure with blood of exactly the same compo-
sition, except that one sample contained a little alcohol and the
other did not, it was clear that in seeking an explanation of the
above results we were limited to two directions: our apparatus
might be imperfect, or the alcohol had a direct action upon the
living organs, heart or lungs, or both.

As regards the apparatus, it was possible that the bottles filled
with alcoholised blood flowed less freely than the others, and thus
cutting off the supply to the heart, gave it less to pump out.

Repeated and most careful examination quite precluded this
explanation. In many cases before commencing an experiment
each of the four Mariotte's bottles was in turn connected with
the vena cava cannula and allowed to pour for a minute into a
beaker, with the invariable result that the quantity collected
from each one did not vary four per cent. from that obtained
from any of the other three. We had in fact taken such care
to have the connections and stop-cocks of each bottle so similar
that a different result could hardly have been possible. In
other cases bottle 1 was first used to feed the heart; then alcohol-
ised blood supplied from bottle 3, with the usual result. The
heart was then recovered by good blood supplied from bottle 2,
and meanwhile bottle 1 emptied of good blood and filled with
alcoholised, its connections being left undisturbed. Then alco-
holised blood from bottle 1 being supplied to the heart, we found

invariably a marked diminution of work, although this bottle had previously, when filled with good (*i. c.* non-alcoholised) blood, kept the heart at full work; and it returned to this standard when subsequently supplied from bottle 3, which meanwhile had had its contents syphoned off and replaced with good blood. An absolutely incontrovertible proof that possible different rates of supply from the bottles had nothing to do with the general result will appear later when we describe the effect of removal of the pericardium.

Once defects of the apparatus were eliminated we had to seek the cause of the result obtained in the heart or lungs. It seemed conceivable (*a*) that the alcoholised blood constricted the pulmonary vessels or otherwise impeded the flow from right ventricle to left auricle ; or (*b*) that it greatly dilated the coronary vessels of the heart and allowed so much blood to be diverted through them as to seriously diminish the proportion of the total amount pumped into the root of the aorta, which was left over to be pumped through the carotid or aortic cannula, with which our outflow tube was connected ; or (*c*) the alcoholised blood might act injuriously on the ganglia and nerves of the heart ; or (*d*) it might act injuriously upon the cardiac muscular tissue.

We were quite at a loss for a time in endeavoring to decide between the above possibilities. At last it was observed that when the heart was supplied with alcoholised blood and this diminished the work done, the organ invariably was much distended, closely filling the pericardiac sac. In the latter a minute hole was always cut as soon as the heart was placed in the warm chamber, to prevent the accumulation of lymph within it, which otherwise is apt to occur ; probably because the efferent lymphatic trunks have been tied or twisted in the operations of isolating the heart and inserting the cannulas. After noticing the expansion of the heart above mentioned, our next experiment was modified by cutting away the pericardium before any observations were made. We then found that even blood containing ½ of one per cent. of alcohol, which had never previously failed to cause a marked diminution in the heart's work, was almost without effect on it. In other cases the experiment was modified by first leaving the pericardium intact and getting the usual alcohol result ; next, recovering the heart by supplying it with good blood ;

then cutting away the pericardium and supplying alcoholised blood from the same flask as before. This now had no effect on the work done by the heart in a minute; though, as will be more precisely stated later, it had a noticeable influence on the bulk of the heart.

Removing the pericardium could obviously have no influence on the rate of supply from our bottles or on the calibre of the pulmonary arterioles; so those possible causes of the general result of the alcohol administration were definitely set aside. It also seemed hardly conceivable that dilatation of the coronary vessels caused the less outflow from the carotid artery or thoracic aorta; for compression of a distended heart by its surrounding pericardium would oppose such dilatation, and the effect ought therefore to be most marked after the removal of that sac, which was exactly the reverse of what we found to occur. That the contractile force of the heart was not directly affected seemed also demonstrated by the very slight diminution of work, if any, which occurred on the administration of alcohol after removal of the pericardium. We thus seemed driven to seek for some alteration in the physical condition of the organ which impeded its action and diminished its work. This alteration was not far to seek. The great swelling of the heart when under the influence of alcohol was obvious. At the height of each systole it nearly filled the pericardiac cavity, and during the diastoles had little opportunity to dilate and receive a fresh supply of blood. Hence the quantity pumped out at each beat became less and less in proportion as the heart swelled. As it seems tolerably certain that the normal heart-beat is of such character that, at the end of each systole, the ventricular cavities are entirely emptied and obliterated, we may state our results as follows: *The action of alcohol administered in the manner and doses above described is, without primarily altering the force of heart-beat, to alter its character, so that the ventricular cavity is not obliterated at the end of systole, and less so the longer the alcohol has been administered. At first this incomplete systole is compensated for by a more extensive diastole, so that the difference between the capacity of the ventricle in complete diastole and that in complete systole remains the same as when the organ was normally beating. Consequently, the quantity of blood pumped*

out at each beat remains as great as before. If the heart be confined in the pericardium it soon, however, ceases to have room to swell during diastole to a size sufficient to compensate for its incomplete systole; and thenceforth, as the swelling increases, the difference between diastolic and systolic capacity becomes less and less. As the necessary result, the quantity of blood pumped round by the organ is proportionately diminished. Removal of the pericardium prevents this result, at least for a considerable time.

Probably the diastolic increase would ultimately, even with the pericardium removed, gain a maximum before the systolic increase of ventricular capacity had reached its limit, if alcohol were administered a longer time, and there would then be a diminution in the blood pumped round; but upon this point we are not prepared at present to make a positive statement. When hearts freed from the pericardium showed a distinct diminution in the work done, we have never been able to obtain any satisfactory recovery; and, as above stated, we are unwilling to lay stress on experiments in which no such recovery was obtained when good blood was substituted for alcoholised.

Gaskell has shown[3] that the heart of the frog and toad can have the extent of its systole or diastole controlled by the vagus nerve. Hence it may be that the characteristic physical change wrought in the muscle of the dog's heart by alcohol is indirectly produced by a primary action of the drug on vagus nerve endings in the organ. Gaskell, himself, however,[4] and Roy,[5] Ringer[6] and others, have found that various substances supplied to the apex of the frog's ventricle bring about a condition of imperfect systole similar to that which we find produced in the dog's heart by alcohol; while other substances exert the reverse effect, bringing the frog's apex into an almost tetanic state of systole. Hence, reasoning from analogy, it is also possible that the alcohol acted directly upon the cardiac muscle. At present we do not find ourselves in a position to decide between these possibilities.*

* This paper was read before the Medical and Chirurgical Faculty of Maryland on April 27, 1883, and an abstract of it published in the *Medical News*, Philadelphia, May 5, 1883. Since the present article was put in type, a paper by Ringer and Sainsbury has appeared in the *Practitioner* for June, 1883. They experimented with various alcohols on the frog's ventricle, and found all stopped the heart in diastole. Their work makes it probable that our results are due to direct action of the ethyl alcohol on the muscular tissue of the dog's heart.

The therapeutical significance, if any, of the results obtained by us we do not feel qualified to discuss; but we may point out that our work seems to show that alcohol should be used with caution in cases of pericardiac effusion, where any increase in the size of the organ, hampered as it is already by the liquid around it, could only be harmful. We trust shortly to investigate the action of other substances upon the isolated dog's heart; especially those substances which have been found to produce dilatation or contraction in the hearts of amphibia and reptiles. If we can establish for the mammal the results which others have obtained on the lower vertebrates, we may perhaps add some little to the knowledge available to the physician in his treatment of the pathological conditions known as dilated and contracted heart.

We append in tabular form the details of some of our experiments. The only point which we think may need explanation is the fact that in some cases arterial pressure is seen to fall while the heart was still pumping some blood up to and out of the outflow orifice, which was maintained at a uniform height. This is due to the fact that the pressure recorded by the manometer depended on two factors: one (the main one), the height of the exit of the outflow tube above the level of the heart; the other, an elastic reaction of the aortic arch and the arterial stumps connected with it, and of the elastic rubber outflow tube, due to the fact that when in good working condition the heart kept them all slightly on the stretch. When the heart pumped less blood this tension diminished or disappeared, and the pressure in the stump of the carotid with which the manometer was connected fell accordingly.

The numbers in the column headed "outflow" give the number of cubic centimetres of blood pumped by the heart through the outflow tube in the minute ending at the time stated in the first column. The figures in the column headed "pressure" indicate millimetres of mercury.

March 12, 1883. Animal under the influence of morphia during the preliminary operation. Heart isolated at 2h. 05m. P. M. Outflow through right carotid. Pressure measured in left carotid.

Time—P. M.	Pressure.	Outflow.	Notes.
2h. 23m.	140	198	
28	138	193	
30	140	197	
36	139	188	
42	141	199	
47	140	190	
2h. 47m. 30s.			Alcoholised blood, 0.25 per cent., turned on.
49	125	118	
51	122	97	
54	120	96	
56	124	103	
2h. 56m. 15s.			Good blood turned on instead of alcoholised.
3h. 01m.	135	142	
06	134	148	Marked recovery.
3h. 14m. 00s.			Pericardium cut away.
21	133	166	
29	140	205	
33	142	203	
36	142	199	
3h. 37m. 30s.			0.25 per cent. of alcoholised blood turned on.
41	135	169	
43	133	161	Pulse slightly irregular.
3h. 43m. 15s.			Good blood turned on.
47	135	168	
53	134	168	The heart now became very irregular and was obviously dying. The experiment, however, shows well enough the comparatively slight action of the alcohol after the removal of the pericardium.

April 26, 1883. Very small dog; under morphia while heart was being isolated. Heart isolated at 2h. 03m. P. M. Outflow cannula in aorta. Pressure measured in left carotid.

Time—P. M.	Pressure.	Outflow.	Notes.
2h. 21m. 00s.	99	140	
23	99	142	
25	99	140	
27	99	145	
30	99	145	
2h. 31m. 00s.			0.25 per cent. alcoholised blood turned on.
33	98	121	
35	98	116	
38	98	100	
41	98	98	
44	98.5	100	
2h. 44m. 20s.			Good blood turned on.
46	99	129	
50	98.5	125	
52	98.5	123	
55	99	122	
57	99	120	
59	98	126	
3h. 00m. 00s.			0.5 per cent. alcoholised blood turned on.
02	96	60	
04	96	28	
06	95	8	
10		0	Pressure rapidly falling as the blood sank in the outflow tube ; not enough being pumped out by the left ventricle to supply the coronary arteries.
3h. 10m. 15s.			Good blood turned on ; the exact moment of turning on the good blood has unfortunately been omitted in the record of the experiment. It was probably at the time here stated, but may have been just before 3h. 09m.
11			A few drops of blood pumped out of the outflow orifice.
13	98	100	
15	98.5	121	
17	98	116	
3h. 19m. 00s.			Pericardium cut away.
22	98	135	
24	98	135	
3h. 24m. 45s.			Alcoholised blood (0.5 per cent.) turned on.
26	98	133	
28	97.5	120	
31	97.5	110	Heart greatly swollen.
33		103	
35	97	105	
3h. 35m. 15s.			Good blood turned on.
37		137	
39		127	
41		128	This experiment shows well the much greater effect produced by the blood containing $\frac{1}{2}$ of one per cent. of alcohol than that containing $\frac{1}{4}$. Also the much less effect of the alcohol in so far as quantity of blood pumped around is concerned, after removal of the pericardium.

May 31st, 1883.   Medium sized dog, etherised while the heart was being isolated.   Heart isolated at 3h. 55m. P. M.   Outflow cannula in aorta.   Pressure measured in left carotid.

Time.	Pressure.	Outflow.	Notes.
4h. 20m. 00s.	102.5	283	
26	103	285	
28	103	273	
30	103	279	
4h. 30m. 30s.			0.5 per cent. alcoholised blood turned on.
32	100	198	
34	98	109	Heart much distended.
36	97	88	
38	96.5	63	The pulse waves on the kymograph tracing have become very feeble.
4h. 38m. 20s.			Good blood turned on.
40	98	98	
42	102.5	264	
44	102	266	
46	102.5	270	
4h. 48m. 00s.			A large slit cut in pericardium.
50	103	283	
53	103	281	
55	102	278	
57	102	277	
4h. 57m. 45s.			0.5 per cent. alcoholised blood turned on.
59	98	180	
5h. 01m. 00s.	99	175	The pericardium was now completely removed, as it was observed that although the ventricles projected through the opening made in it, the auricles, especially the right, were compressed and impeded in their diastole.
05	101	260	
07	100.5	245	
09	100	241	
5h. 12m. 30s.			Good blood turned on.
15	103	295	
17	102.5	278	
19	102.5	284	The experiment was stopped here, with the heart and lungs still in good condition. On the whole, it is one of the most satisfactory in our series, as the lungs remained in good order throughout, instead of becoming œdematous towards the end of the experiment, as they usually do, impeding the blood flow and more or less vitiating the result. This gradually increasing pulmonary œdema is one reason why we have rejected all experiments but those in which the heart showed decided recovery after the removal of the alcoholised blood ; it is, also, we feel sure, mainly responsible for our failure in most cases to get a complete recovery of the organ as indicated by the outflow.

February 26th, 1883.   Animal under morphia while heart was being isolated.   Isolation completed at 1h. 05m. P. M.   Outflow cannula in right carotid.   Pressure measured in left carotid.

Time—P. M.	Pressure.	Outflow.	Notes.
1h. 42m. 00s.	122	204	Pericardium removed before the first measure-
45	121	202	ment of outflow was made.
48	123	207	
1h. 50m. 00s.	119		0.25 per cent. alcoholised blood turned on.
52	119	200	
54	117	184	
56	117.5	183	
1h. 57m. 00s.			Good blood turned on.
59	116	183	
2h. 13m. 00s.	122	198	Three measurements made between 1h. 59m.
16	122	203	and 2h. 13m. were thrown aside as useless,
21	122	200	on account of the discovery of a bubble of
26	119	202	gas imprisoned in a bend of the supply tube
			of the Mariotte's bottle. This greatly di-
			minished the quantity of blood reaching the
			heart. Another bottle having been con-
			nected with the heart, the gas was removed
			and the experiments continued.
2h. 27m. 00s.			0.25 per cent. alcoholised blood turned on.
29	118	197	
32	120	198	
2h. 33m. 00s.			Good blood turned on.
36	126	210	
2h. 38m. 00s.			0.5 per cent. alcoholised blood turned on.
40	120	202	
42	123	205	
45	122	205	
2h. 46m. 00s.			Good blood turned on.
50	123	200	
2h. 51m. 00s.			1 per cent. alcoholised blood turned on.
53	119	195	
55	117	192	
57	117	190	
59	118	191	
3h. 00m. 00s.			Good blood turned on.
03	121	203	
07	123	208	
			Thoughout this experiment the lungs kept in
			good condition. It shows very well the
			slight effect of alcohol on the quantity of
			blood pumped out by the heart when the
			pericardium has been removed. Even blood
			containing 1 per cent. of alcohol had very
			little influence in diminishing the outflow.

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THE DIRECT INFLUENCE OF GRADUAL VARIATIONS OF TEMPERATURE UPON THE RATE OF BEAT OF THE DOG'S HEART.

By H. NEWELL MARTIN, M. A., M. D., D. Sc., Professor in the Johns Hopkins University.

(Abstract.)*

In the investigations described the method of experiment was such as to completely isolate physiologically the heart of the dog from all the rest of the body of the animal, lungs excepted.

This was accomplished by occluding the right and left carotid and subclavian arteries, the aorta just beyond the origin of the left subclavian, and ligaturing both venæ cavæ and the azygos vein. In consequence the only fraction of the systemic circulation left open was that through the coronary system of the heart: no organ but the heart itself has any blood sent it, except the lungs. Hence the cerebro-spinal nerve-centres and the sympathetic ganglia very soon die, while the heart remains alive, in good working condition, for two hours or more. The right auricle is supplied uniformly with defibrinated calf's blood, conveyed to the superior vena cava from Mariotte flasks. The blood, after traversing the pulmonary circuit, is finally pumped by the left ventricle into a cannula, which is tied into the aorta just beyond the origin of the left subclavian artery. From the distal end of the cannula a wide rubber tube carries the blood to an exit cannula seven or eight feet above the level of the heart. By raising or lowering this exit, and by raising or lowering the level of the Mariotte flasks feeding the heart, arterial and venous pressures could be changed at will, or maintained very nearly constant.

Venous and arterial pressures being kept constant, the temperature of the blood supplied to the heart was gradually changed by raising or lowering the temperature of the water

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* Reprint from Proc. Roy. Soc. No. 223, 1883. This paper will shortly be published in full in the Philosophical Transactions, as the Croonian Lecture for the year 1883.

contained in the vessels in which the feeding Mariotte flasks were immersed.

The pulse-rate was recorded by a Fick's spring manometer, and arterial pressure by a Marey's mean-pressure mercury manometer, each being connected with the central stump of a carotid artery. Temperatures were read by means of a thermometer tied into the root of the left subclavian, so that its bulb projected into the aortic arch.

Uniform artificial respiration was maintained.

As the result of many experiments it was found (1) that the isolated dog's heart beats quicker when supplied with warm blood, and slower when cold blood is supplied to it; (2) that the rate of beat depends much more upon the temperature of the blood in the coronary arteries than on its temperature in the right auricle or ventricle; (3) that when defibrinated calf's blood is used to feed the heart, that organ cannot be kept alive as long as when defibrinated dog's blood is employed; (4) that no matter how long an experiment lasts, the defibrinated blood, circulated again and again through heart and lungs, shows no tendency to clot; hence fibrinogen is not produced in those organs.

The question answered by the first of the above results was the one for whose solution the research was undertaken. The experiments show that, in spite of its highly-developed extrinsic nervous apparatuses, the heart of the mammal does, so far as its rhythm is concerned, in its own nervo-muscular tissues, respond to temperature variations within wide limits (42°—27° C.), just as the frog's heart or that of the embryo chick does. To account for the quick pulse of fever we, therefore, need not look beyond the mammalian heart itself; we require no theoretical assumption of any paralysis of inhibitory, or any excitation of accelerator cardio-extrinsic nerve-centres.
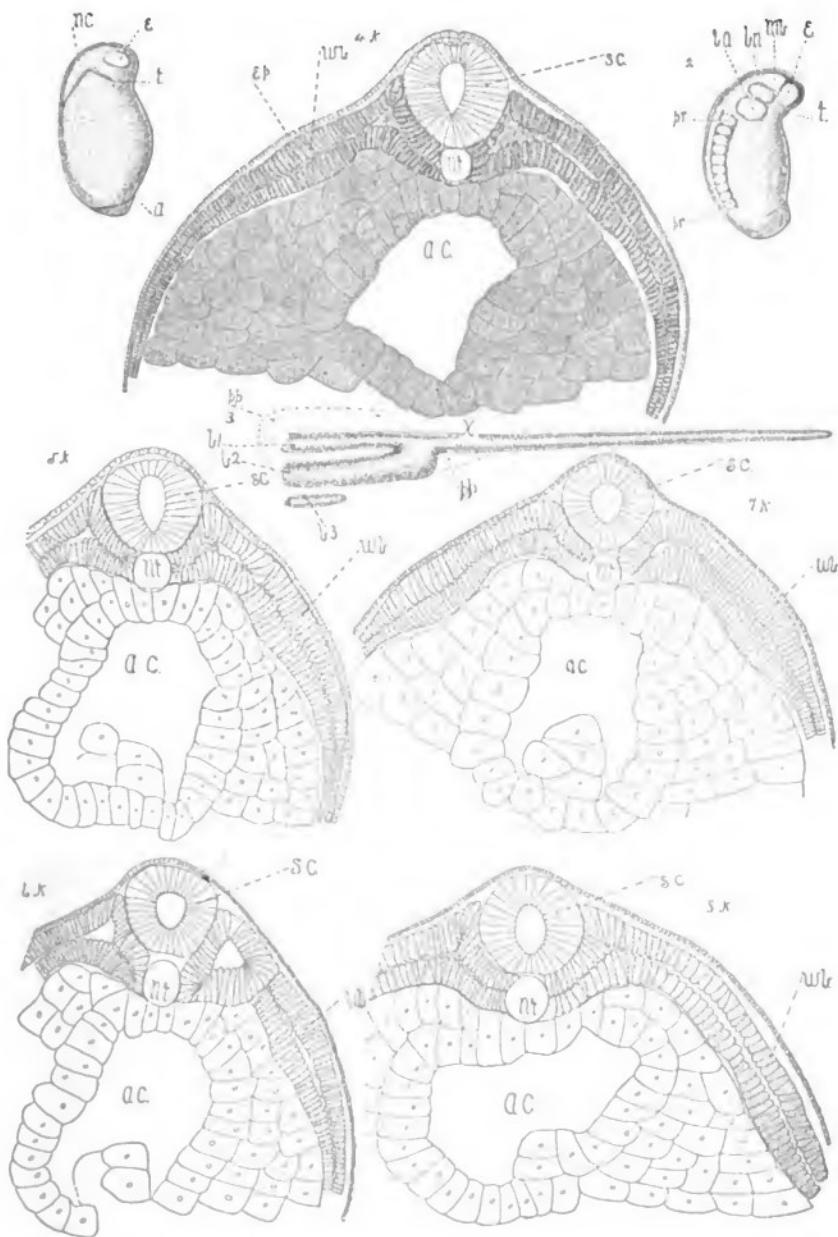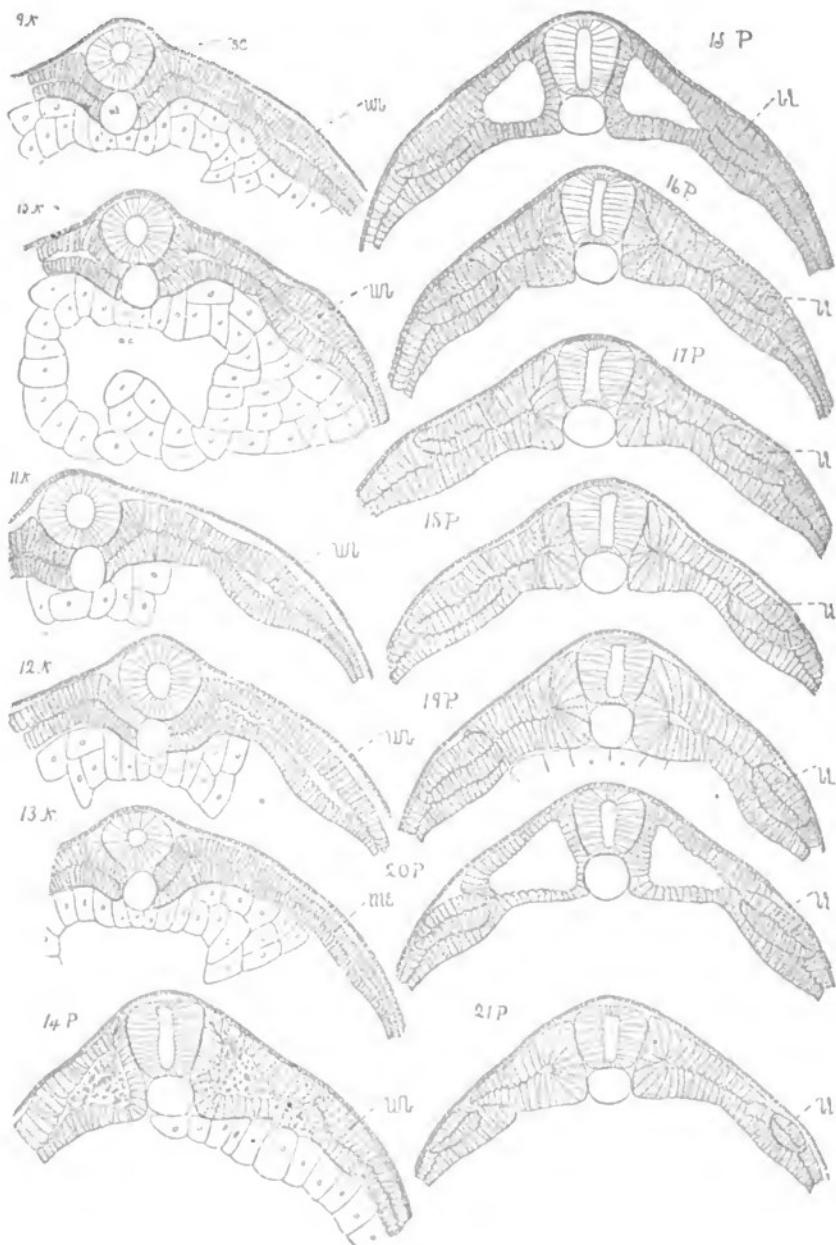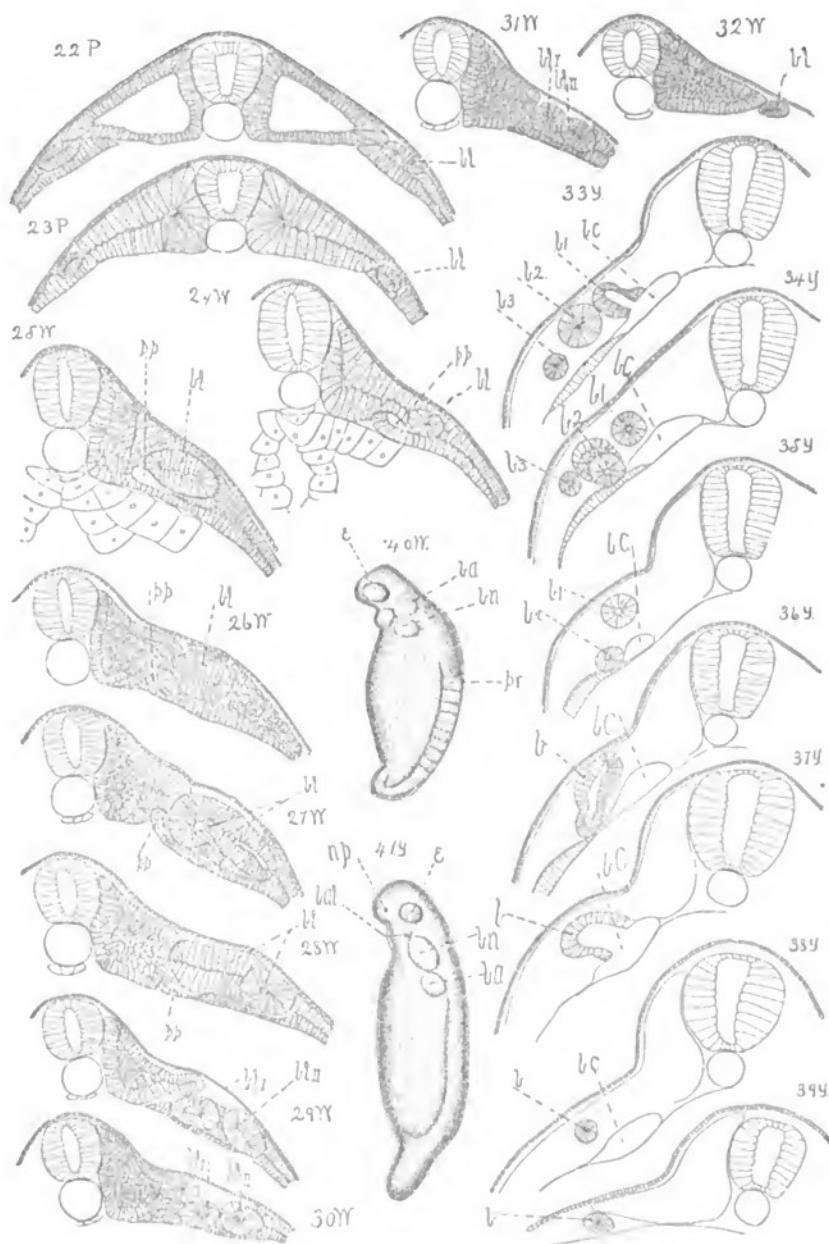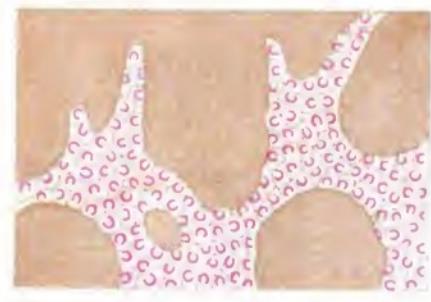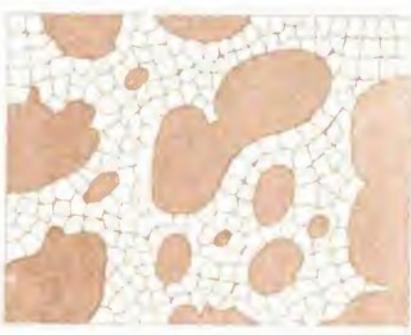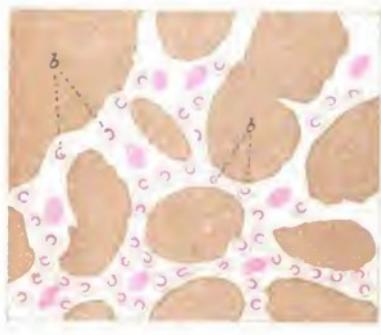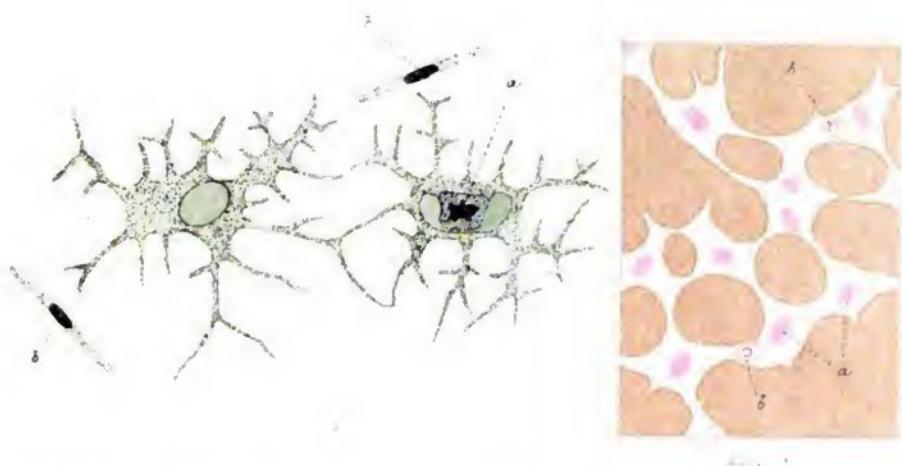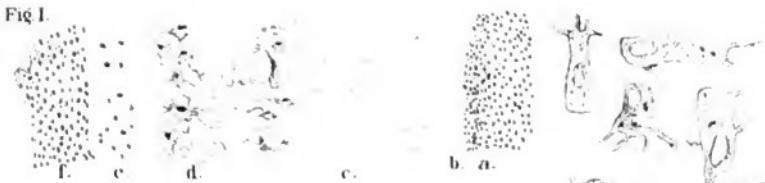
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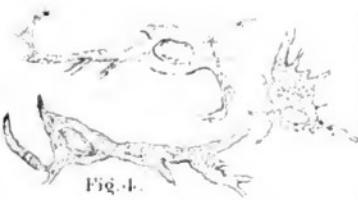
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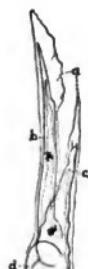
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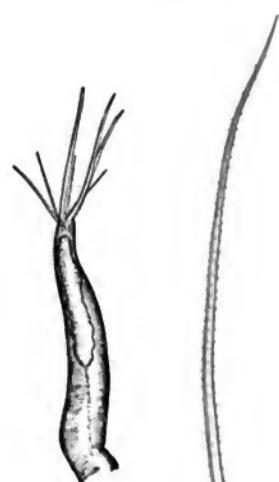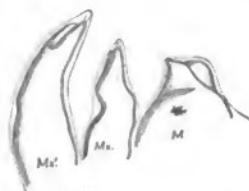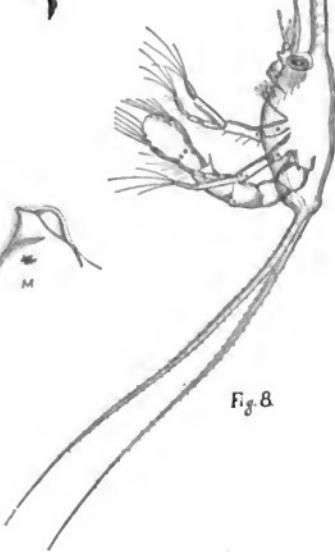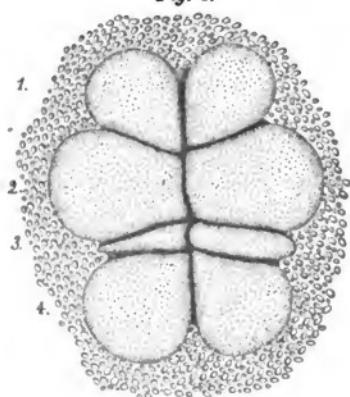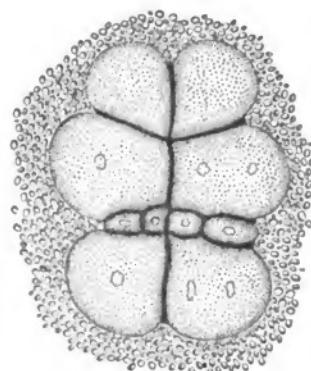
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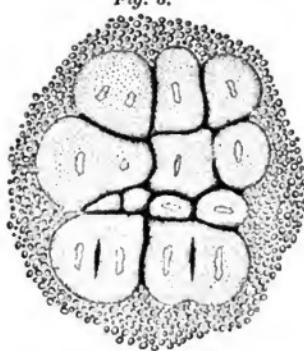
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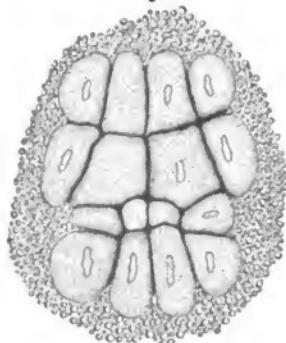
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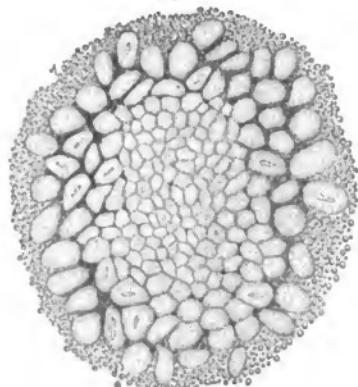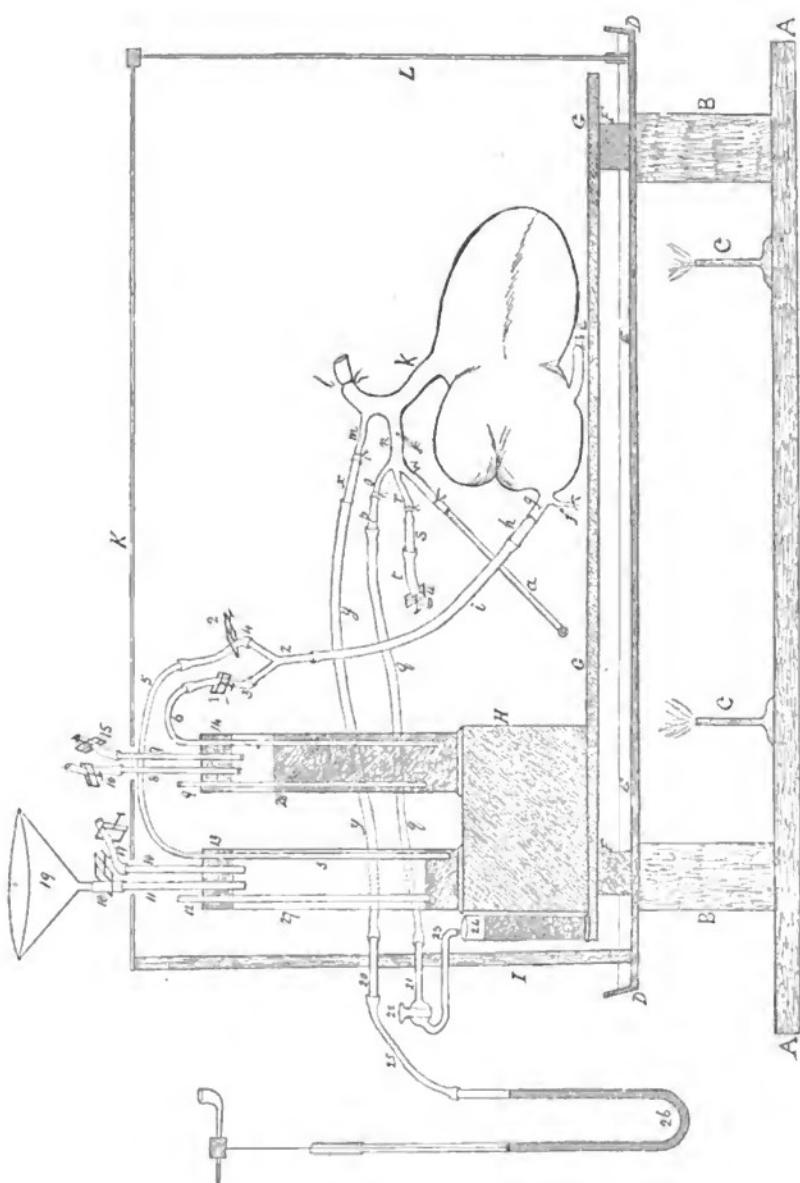
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Plate IX.

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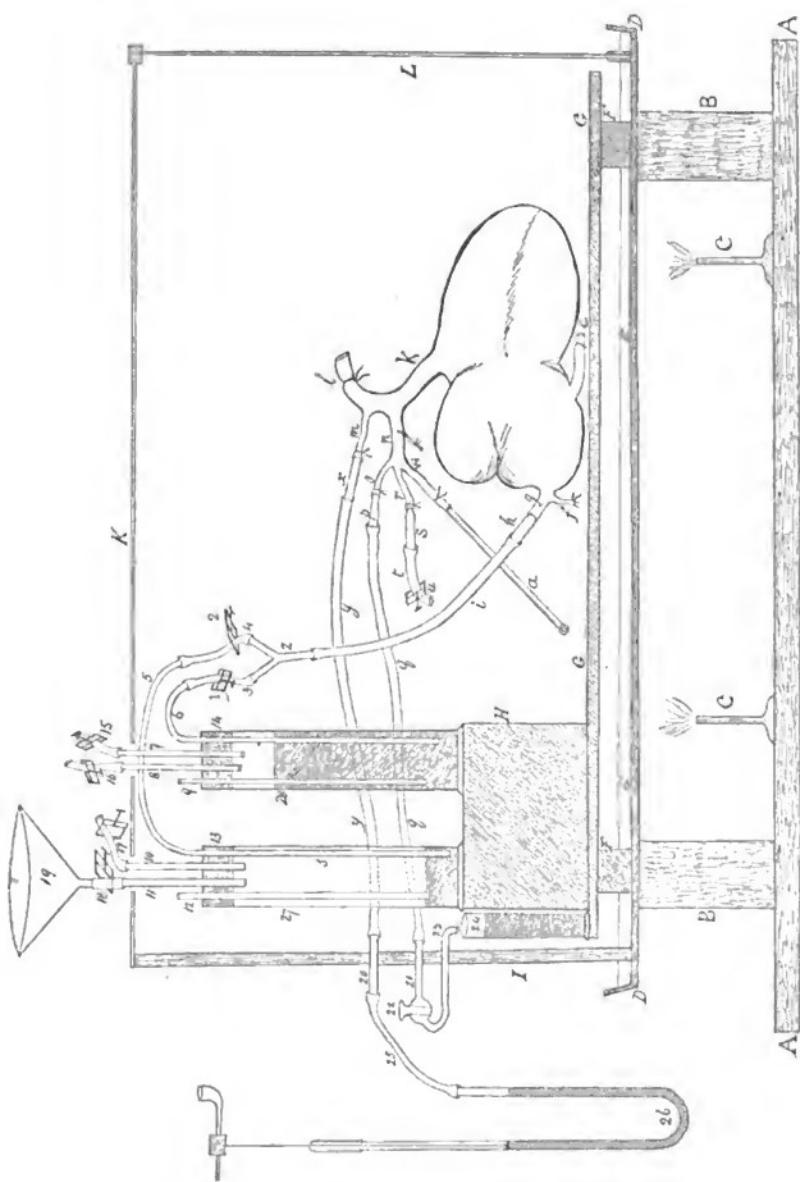
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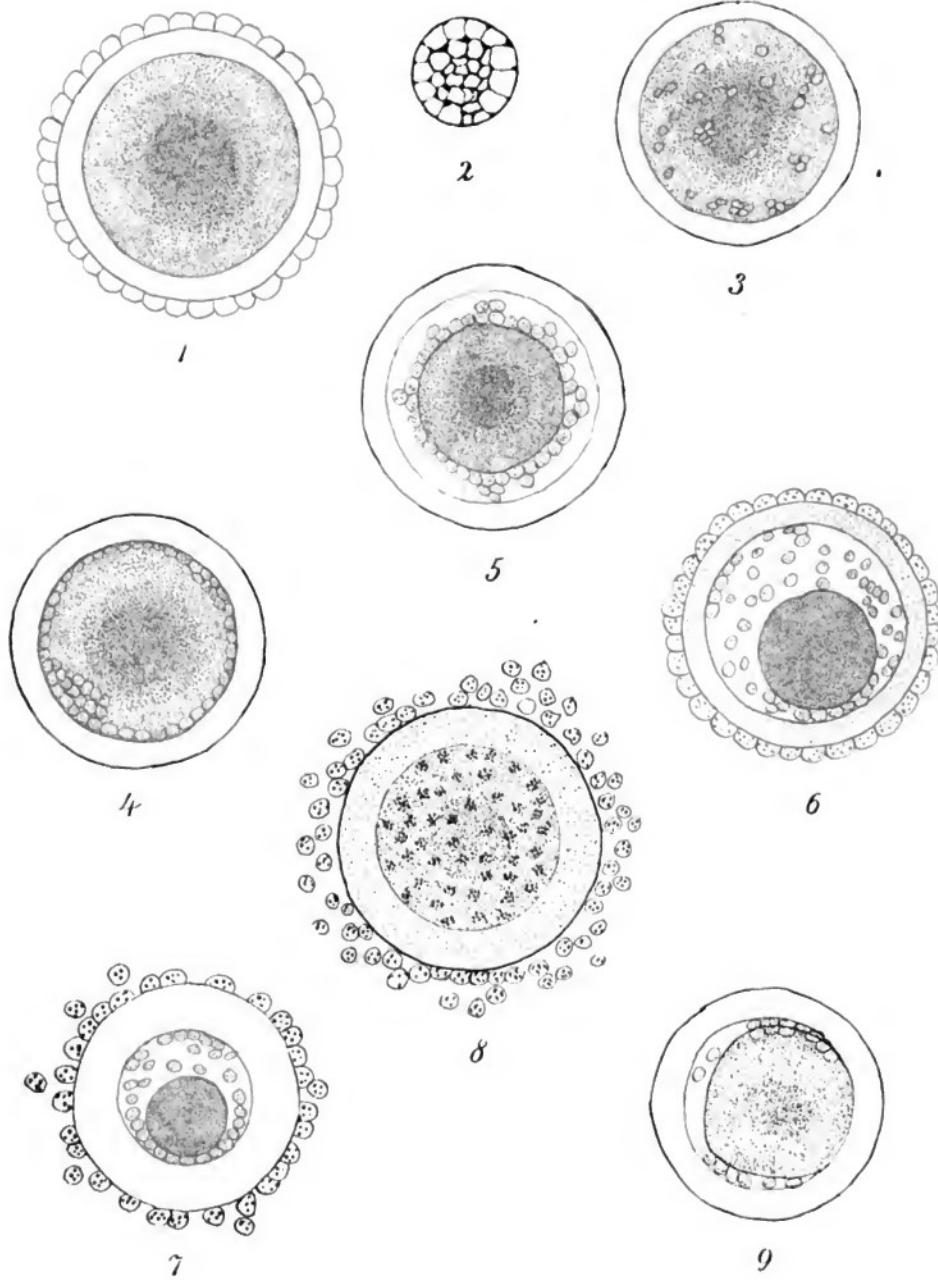
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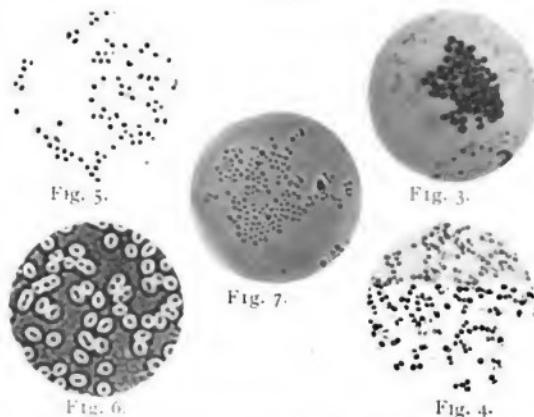
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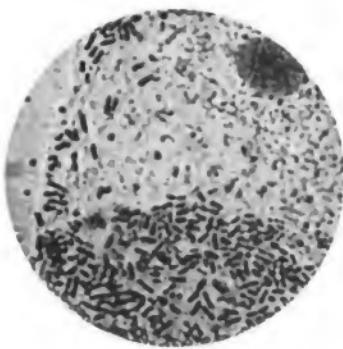
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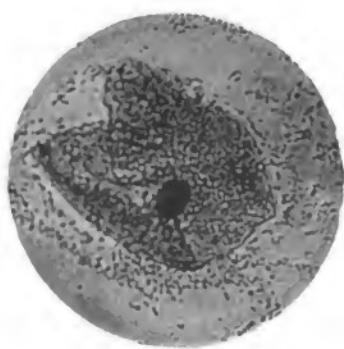
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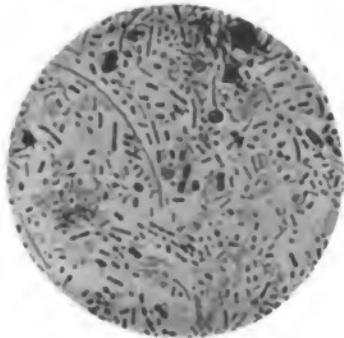
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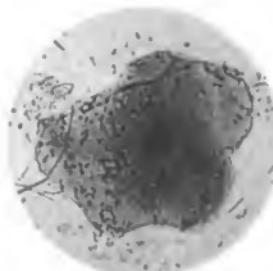
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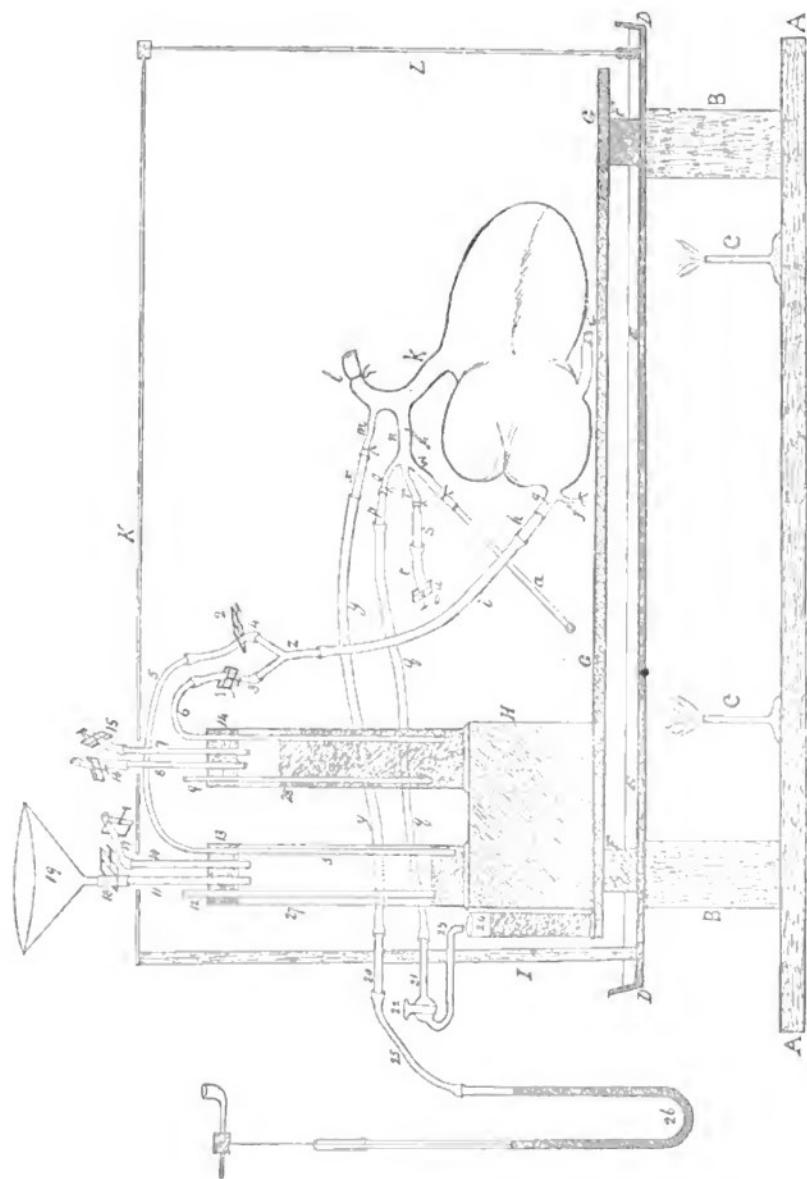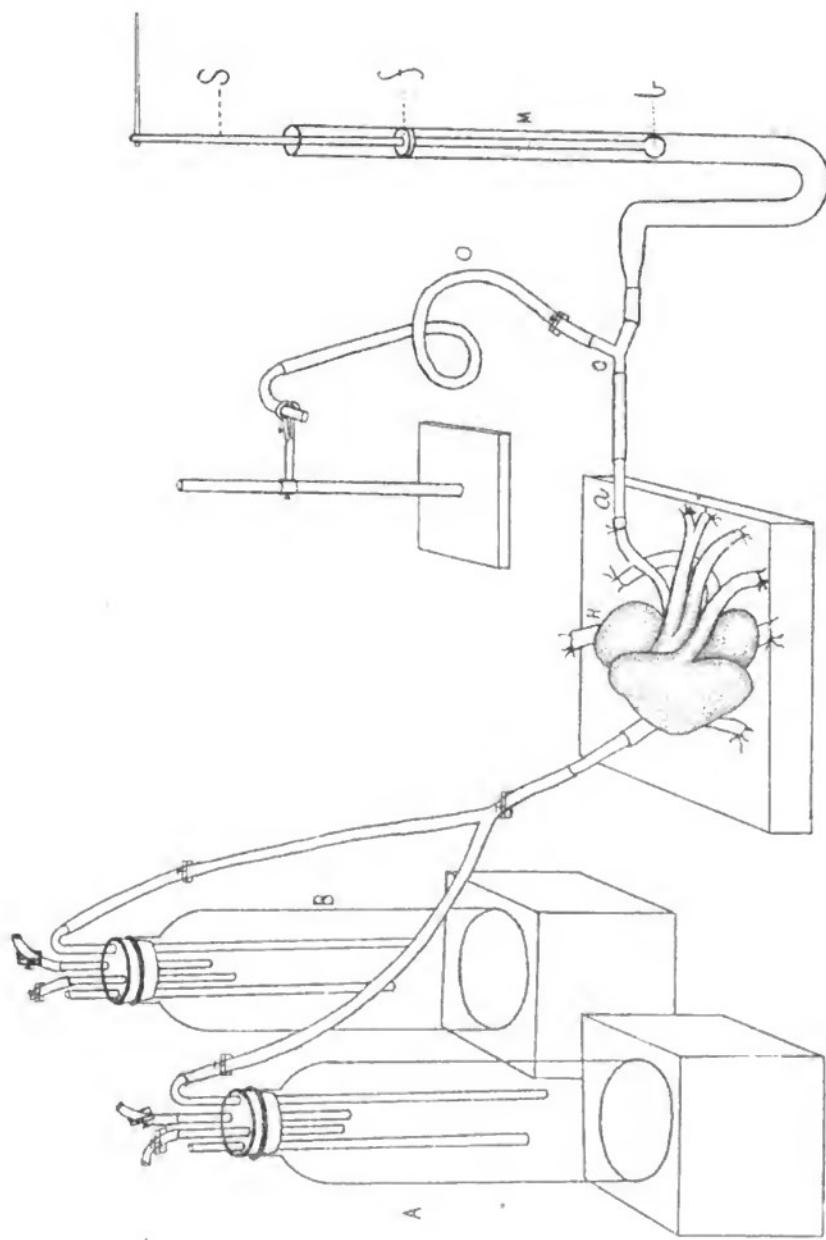
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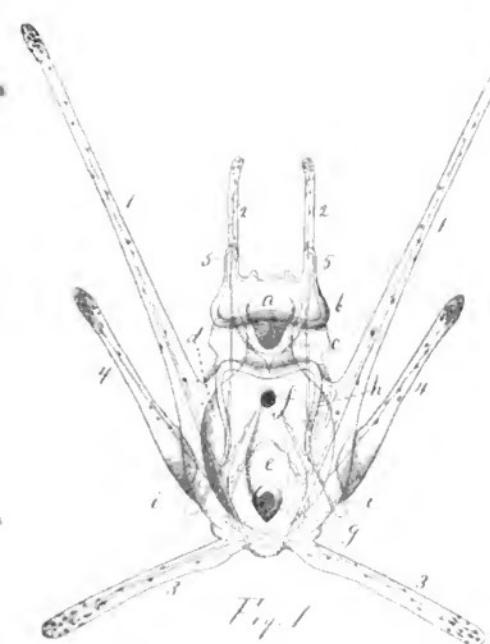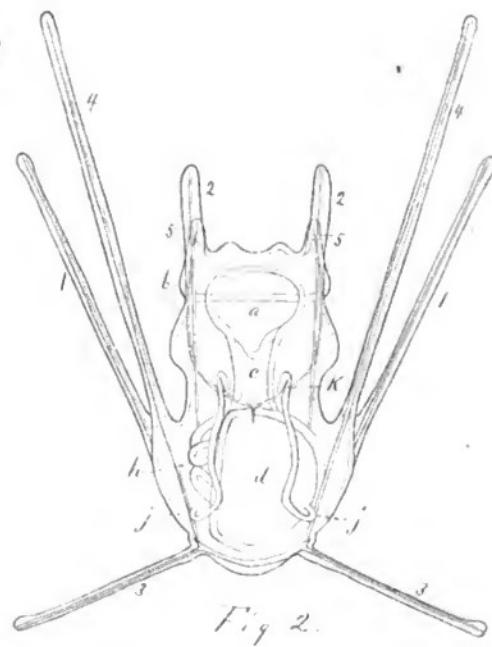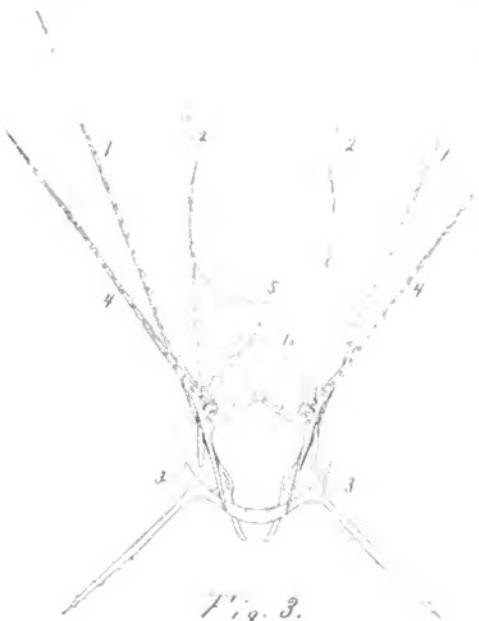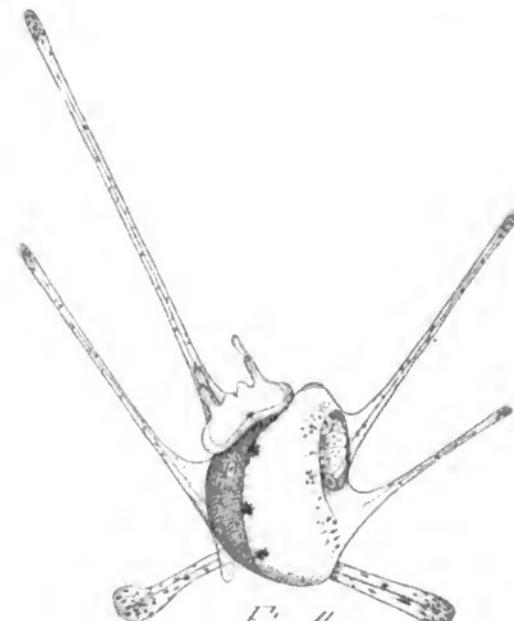
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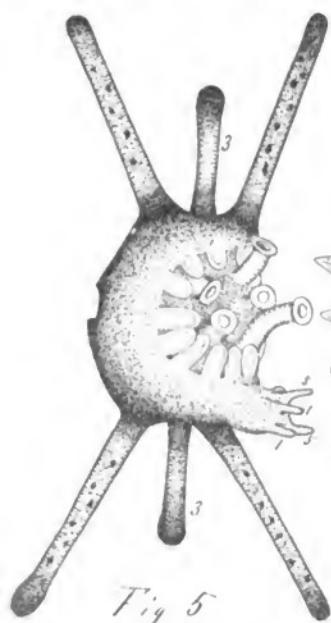
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Fig. 6.

Fig. 12.
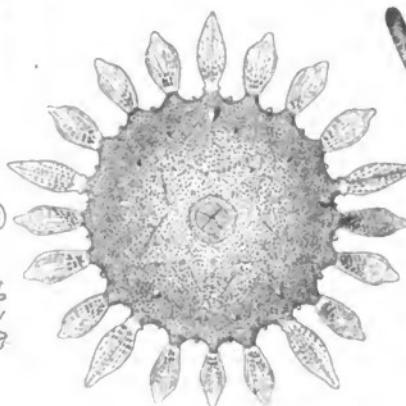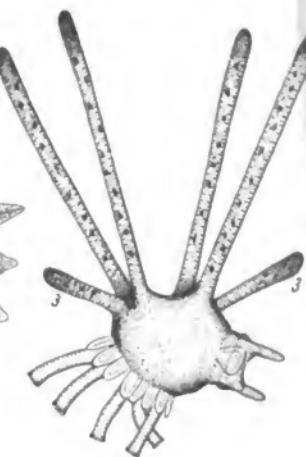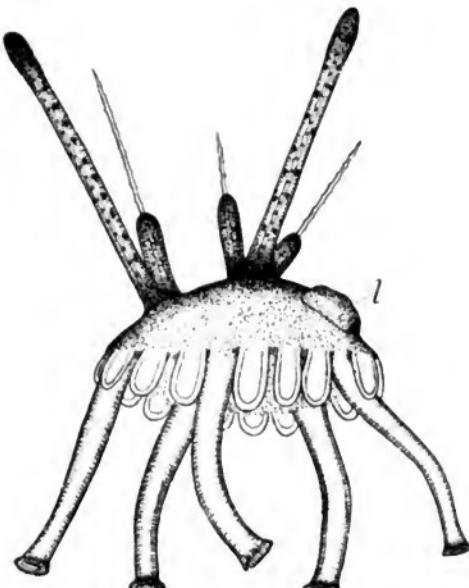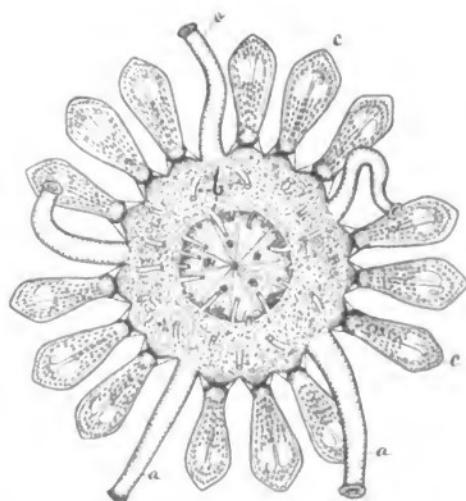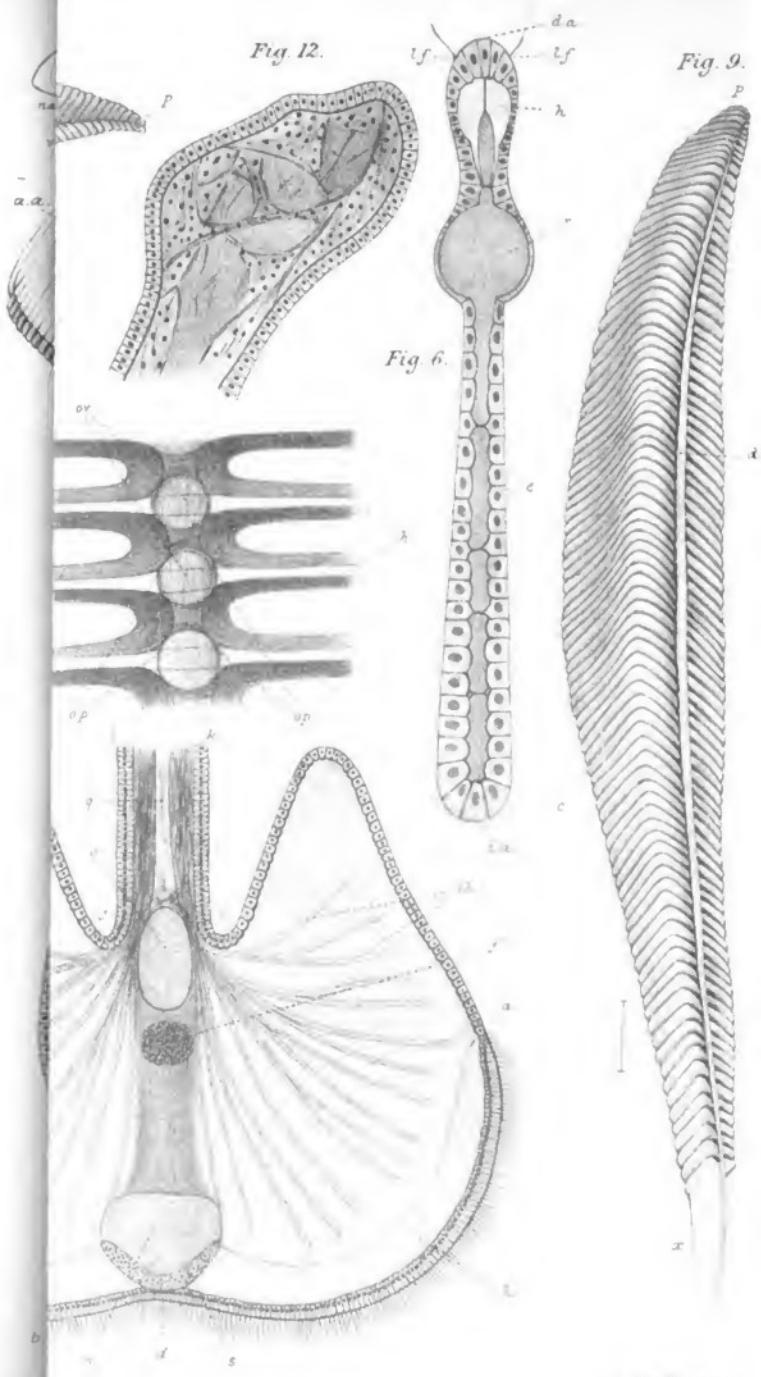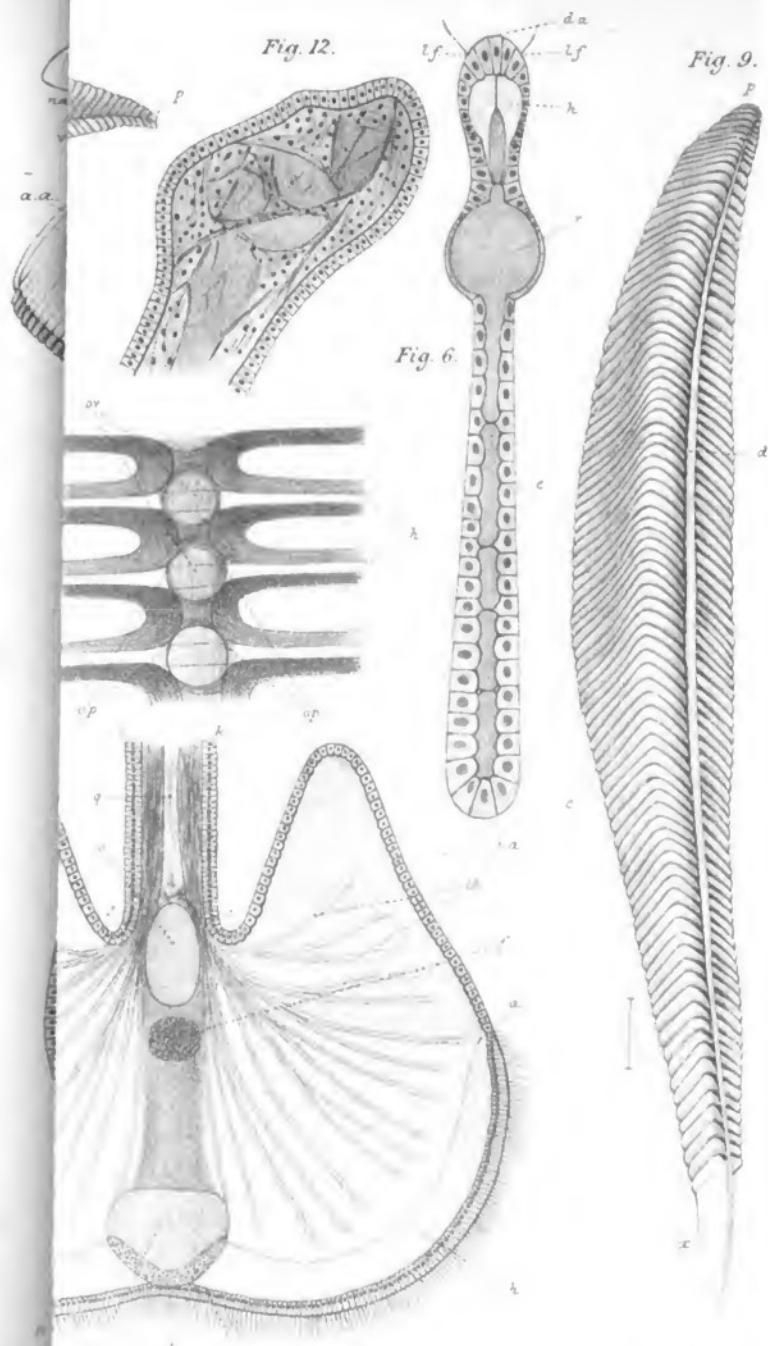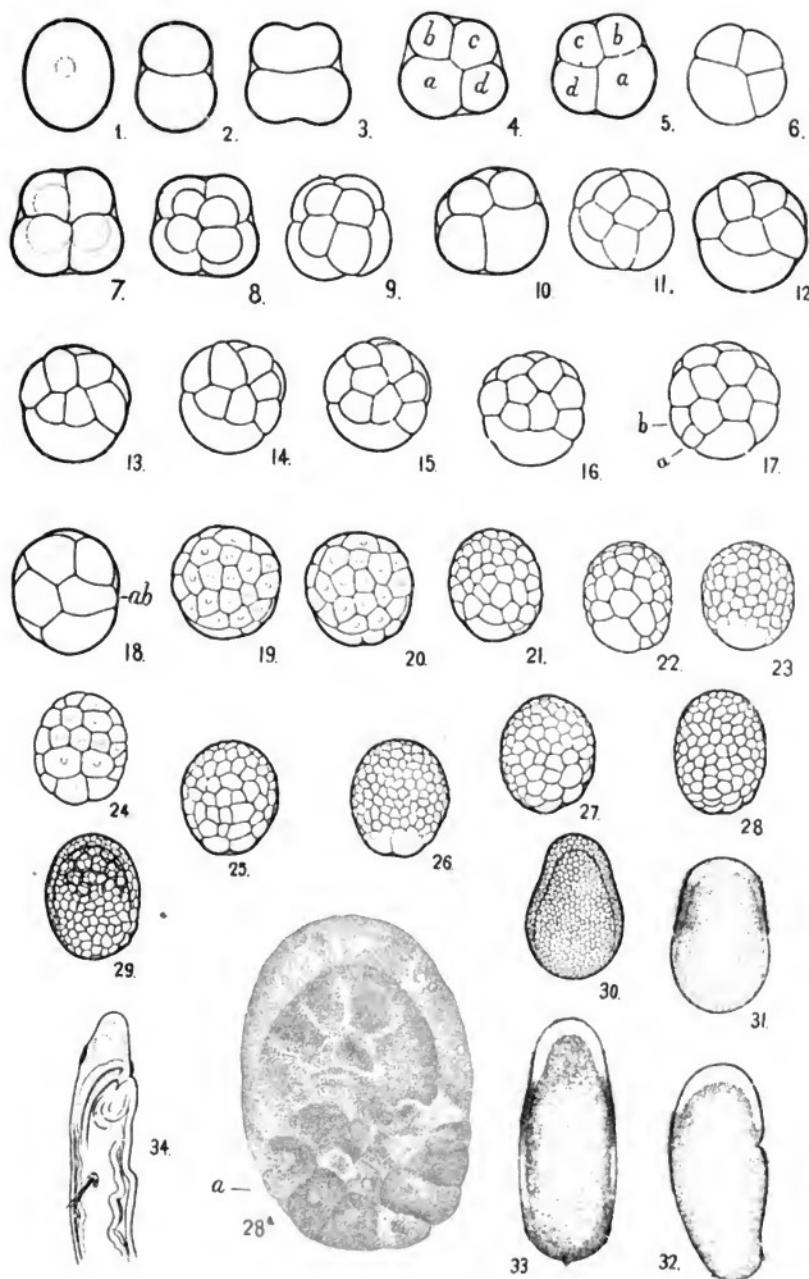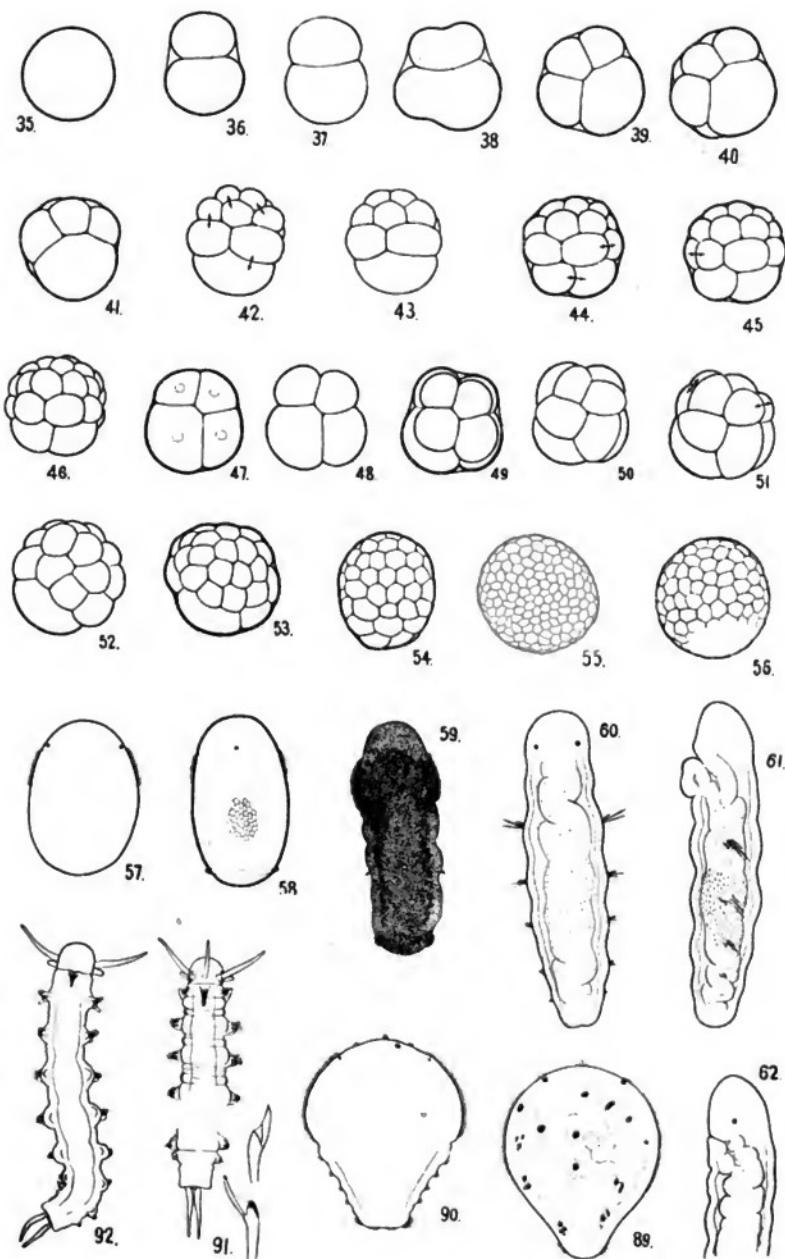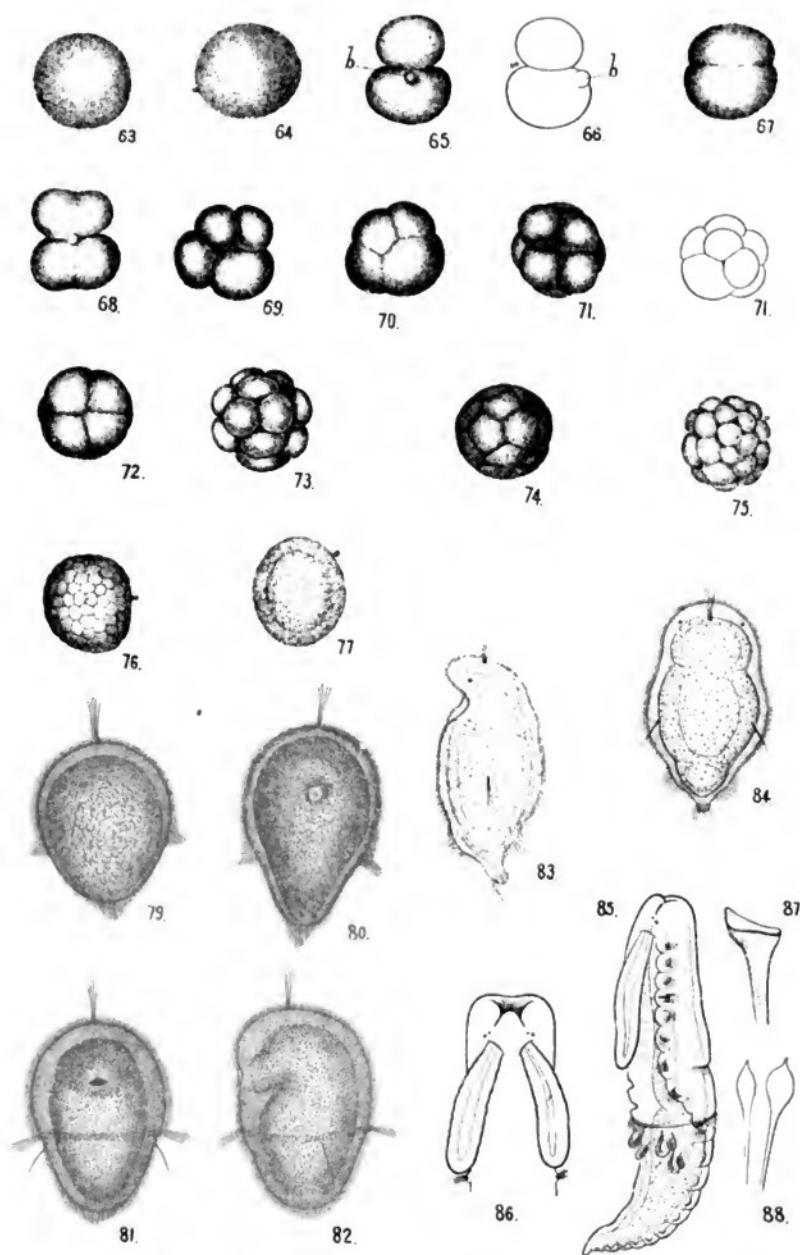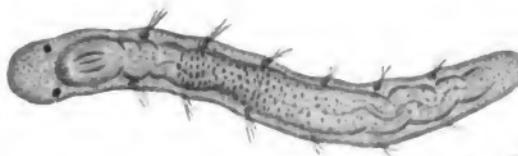
Fig. 9.

Fig. 6.

Fig 5

Fig.10.
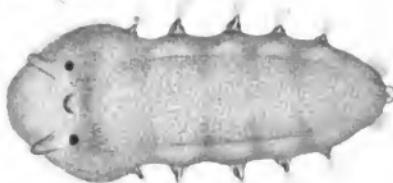
Fig 9
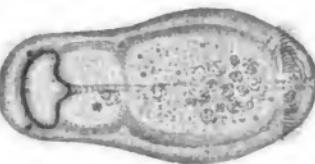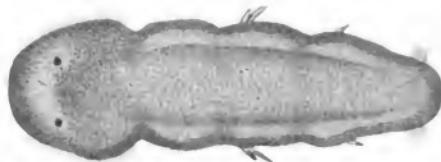
Fig. 4.

Fig 8

Fig. 3.

Fig 7

Fig. 2.

Fig 6

Fig 1

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

W. K. B.

6.

7.

1.

2.

3.

a —

4.

5.

Fig. 1.

Fig. 2.

Fig 3.

Fig 6

Fig. 4

Fig. 5

100μ